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NEWS AND NOTES

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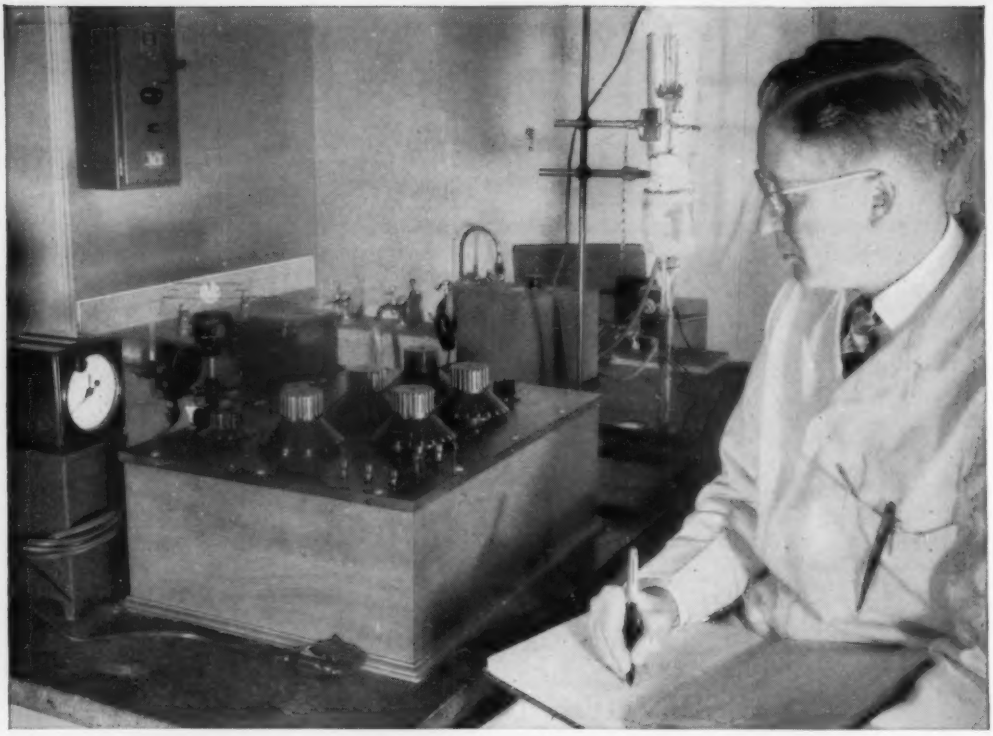
VOLUME 112, NUMBER 2918

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE



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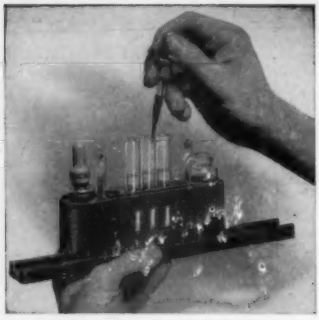

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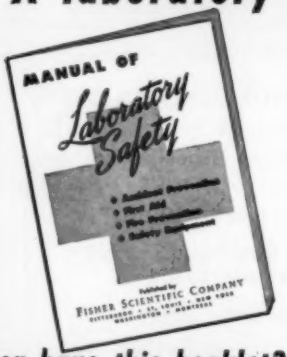

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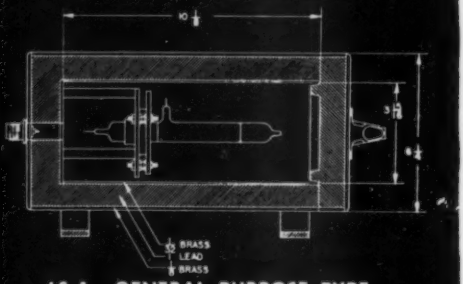

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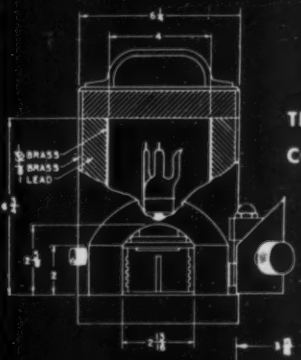

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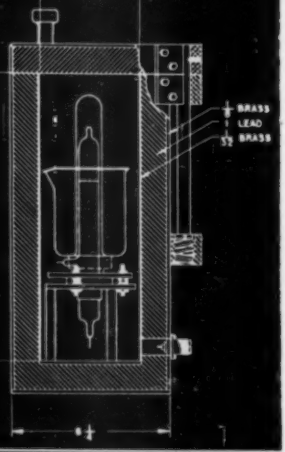

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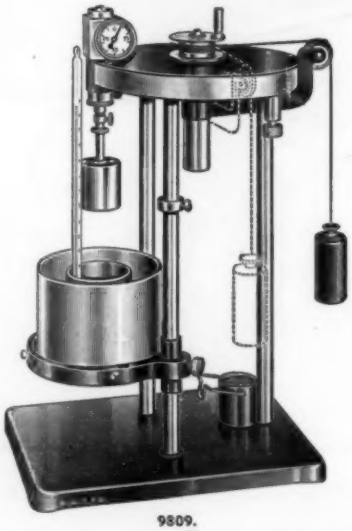

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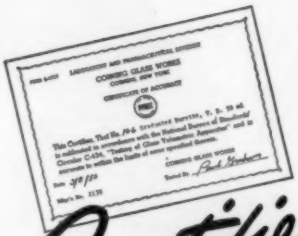

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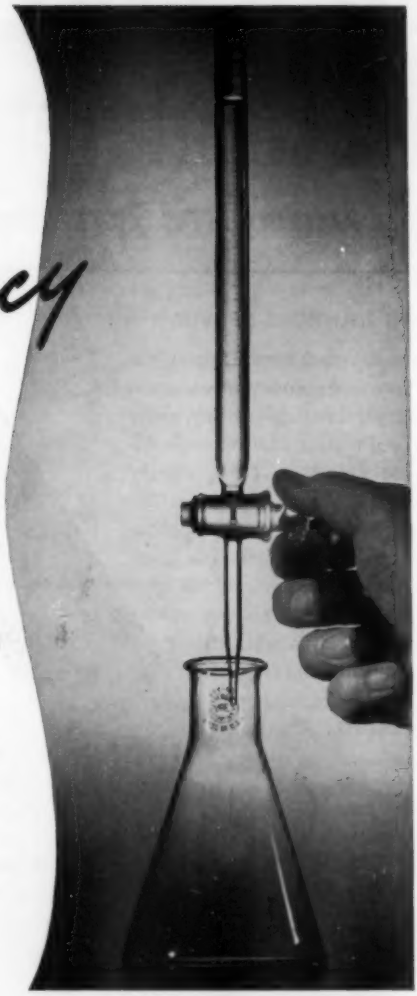

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Protein and Nucleic Acid Conference

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A Gordon Research Conference on "Physical Methods in Nucleic Acid and Protein Research" was held at New Hampton, New Hampshire, August 28 to September 1, 1950. The conference had a marked international character, largely in consequence of support from the Rockefeller Foundation. Ten European scientists were numbered among the seventy participants.

Broadly speaking, the major theme was the interaction of radiation with proteins and nucleoproteins *in situ* and *in vitro*. The development included electron microscopy; x-ray diffraction; ultraviolet and infrared spectroscopy, microspectroscopy, and microscopy; and photochemistry. The minor theme was developed in a symposium on the molecular size and shape of desoxypentose nucleic acid and several papers on recent ultracentrifugal research.

Two papers on electron microscopy demonstrated the striking progress that is being made in the study of viruses in the host and in the photography of protein molecules. R. W. G. Wyckoff (USPHS), in a brilliant series of photographs, showed the various stages of bacterial cells undergoing attack by bacteriophage (Figs. 1 and 2). With the aid of thin sectioning, it was quite readily demonstrated that bacteriophage proliferation takes place throughout the cell, not on the surface. On the other hand, the proliferation of influenza virus in infected tissue occurs at the edge, with filaments growing out. Considerable evidence of the linear aggregation of the spherical influenza virus was demonstrated. Finally, from the work of Dr. Wyckoff and Kenneth Smith, photographs were shown of the polyhedral bodies which form in such large numbers in virus infections of insects. These bodies are shown to be crystalline inclusions with virus particles readily discernible on the surface. In some cases a membrane is found surrounding the polyhedral bodies, and a marked irregularity occurs near the center of the body. These polyhedral bodies develop in the nucleus, not in the cytoplasm.

C. E. Hall (MIT) stressed the difficulty of identifying macromolecules that are readily discernible with a resolution of 30 A. Ambiguities are most readily eliminated by restricting examination to crystals, where the periodicity is a certain check on the macromolecule being viewed. By means of the shadow transfer replica technique, photographs slightly out of focus reveal the individual molecules in the crystal faces of many protein crystals. Edestin is found to be face-centered cubic, with a unit cell dimension of 114 A and a molecular weight of 300,000. Two of the three unit cell dimensions for canavalin are found to be 104 A and 68.5 A. Catalase is essentially orthorhombic, with unit cell dimensions of $64 \times 64 \times 159$ A. When these objects are brought further into focus, the periodicity becomes less discernible, but fine structure on the surface of individual molecules then appears. It is not yet clear whether these are characteristic of the native molecule or are the result of mild denaturation occurring during specimen preparation.

The dominant role of the α-keratin configuration in the molecular architecture of globular proteins gains further support as more structural information becomes available. W. T. Astbury (Leeds) described recent work on the x-ray diagrams from bacterial flagella prepared according to Sven Gard. These flagella, which are rodlike, with dimensions of about $120 \text{ A} \times 20{,}000$ A, are among the simplest systems concerned primarily with producing movement and may be the most elementary manifestation of muscular activity. It is most interesting to find that films produced from these flagella show fine α-keratin reflections and, upon squeezing, this goes over into the β-keratin pattern. With respect to x-ray studies of nucleic acid, it appears that no photographs show any more reflections or detail than those taken in 1937. The original idea still prevails that details revealed by these photographs, together with the extraordinary high density of nucleic acid, point to a structure for the molecule in which the nucleosides have a parallel platelike arrangement perpendicular to the phosphate ester chain. I. Fankuchen (Polytechnic Institute of Brooklyn) gave a critical review of the interpretation of single crystal photographs.

The second day of the conference was devoted almost entirely to ultraviolet microscopy and microspectroscopy. The session was opened by T. Caspersson (Stockholm), who presented a masterful account of the problems inherent in quantitative ultraviolet microspectroscopy of tissues. Professor Caspersson pointed out that for quantitative work all the light from the object should be gathered by the microscope objective and that, therefore, it is necessary to use microscope objectives having numerical apertures of at least 0.85 and to take into due account the pos-

sible scattering losses caused by the variations in refractive index of the material being examined. He also stated that a polarizing prism has been in use in his ultraviolet microspectroscopic apparatus since 1940, and that the effects that have been observed were of the order of 0.1–1.0% in tissues. Such effects are very hard to measure accurately, although some attempts, particularly in connection with the protein absorptions, are now being made in that direction. A promising new development seems to be the use of x-ray absorption procedures as developed by Engstrom, to determine the mass distribution in tissues.

Following the above presentation, a symposium on ultraviolet microscopy was held. Almost all the participants described their work in using one or another kind of reflecting microscope objective. There seemed to be no question about the general utility of this type of microscope objective for microspectroscopic work. R. Barer (Oxford) described the work he has been doing with E. R. Holiday, using a Burch reflecting objective (N.A. 0.65) that has the large mirror aspherized. The arrangement for microspectroscopic studies makes use of a logarithmic cam to advance the plate holder. Dr. Barer also described a scheme he has been working on for the cathode-ray oscilloscope presentation of the ultraviolet absorption spectrum of a single cell. R. C. Mellors (Sloan-Kettering Institute) described various ultraviolet microspectroscopic arrangements that make use of the spherical reflecting and reflecting-refracting microscope objectives now available in this country. He outlined a possible application of such techniques to problems connected with cancer diagnosis. J. T. Randall (Kings College, London) described two-mirror nonconcentric spherical microscope systems now being developed in his laboratories by Wilkins and Seeds. If the two mirrors are kept nonconcentric, it seems possible to construct a series of microscope objectives of different numerical aperture which appear to have utility for certain spectroscopic applications. By the addition of an Amici component the system is no longer achromatic, but numerical apertures as high as 1.4 have been realized.

FIG. 1. Electron micrograph of a section through part of a single epithelial cell of chorioallantoic membrane of chick embryo diseased with vaccine virus. At bottom of cell the newly formed elementary bodies of the virus (about 5 mm in diameter each) stand out clearly from the remains of the cytoplasm in which they have developed. At the top of the cell the elementary bodies are more faintly seen embedded in the less altered protoplasm. ×8600 (approx.).

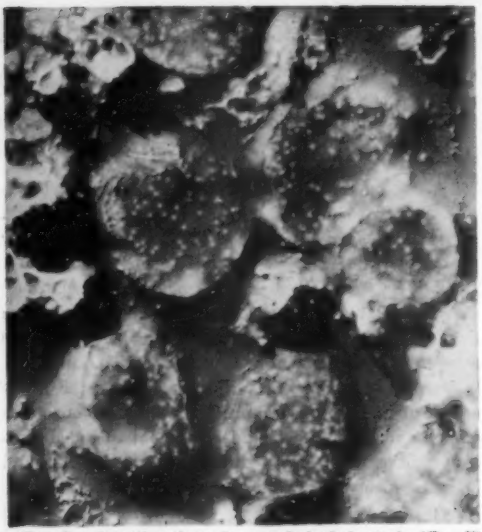

FIG. 2. A section through several single bacteria (*E. coli*) that have been infected with the T_2 strain of bacteriophage. The new particles of bacteriophage developing throughout the bodies of the bacteria are clearly visible as dots slightly more than 1 mm in diameter. ×9000 (approx.).

A. W. Pollister (Columbia University) emphasized the revolution in cytology that has been initiated by the introduction of the reflecting microscope, together with the ultraviolet and infrared investigations that it makes possible. With this in mind he described the work he has done on estimating nucleic acids by a combination of ultraviolet microscopy and quantitative staining reaction. B. Commoner (Washington University) described the measurement of ultraviolet spectra of structures in intact living plant cells. By means of a microspectrophotometer employing a Grey reflecting objective, ultraviolet (and visible) absorption spectra of areas and pigments in living plant hair cells have been obtained. Such spectra are difficult to interpret because they represent the added

absorption of the vacuole fluid and the surrounding protoplast layer. By measuring a second absorption spectrum through another thickness of the same cell, however, it is possible to calculate the separate absorption spectra of vacuole and protoplast. The protoplast spectrum shows a sharp maximum at 280 mμ and one at 340 mμ. The separate ultraviolet absorption spectra of living plant cell nuclei have been obtained by similar measurements. Dr. Commoner emphasized that the absorption spectra of intact cells cannot be properly interpreted from measurements made at a single point in the cell.

The session was concluded with fine papers by J. F. Scott (MIT and Massachusetts General Hospital) and A. D. McLaren (Brooklyn Polytechnic). Dr. Scott pointed out that certain purines and pyrimidines, as well as some simple amino acids, show a considerable sharpening of their absorption spectra, and an increase in the fine structure in the ultraviolet region, when films of such materials are cooled to very low temperatures. Unfortunately, the application of such techniques to intact biological systems such as tissues appears to be limited, since the more complicated molecules such as nucleotides, nucleic acids, and proteins generally show very little sharpening or increase in fine structure of their ultraviolet absorption bands when cooled. Correlation of such data, however, with that from infrared measurements for simple molecules may lead to interesting structural information. This work was done in collaboration with J. R. Loofbourow (MIT).

Dr. McLaren discussed his recent work on the photochemistry of proteins, enzymes, and viruses. Of potential interest to cellular biologists investigating the structure of living tissues with ultraviolet light microscopy is the influence of ultraviolet light on cellular constituents. In view of the inactivation-denaturation of enzymes and related substances, it is obvious that, in the act of observing living tissue with ultraviolet light, the tissue undergoes degradation as a result of photolysis. The quantum yields for inactivation are low and are lower the greater the molecular weights of the substances involved. Whether the quantum yields are sufficiently large seriously to affect observations on cellular constituents with ultraviolet light remains to be determined.

In model experiments performed with phenyl-substituted alkyl amides, an increase in the photochemical effect was noted when there were three carbon atoms between the phenyl group and the amide group, as compared with the effect noted when there were two, one, or four carbon atoms intermediate between these two groups.

The third day of the conference was devoted entirely to infrared studies. G. B. B. M. Sutherland (University of Michigan) started the session with a lucid talk describing the principles governing the infrared method of structural analysis. The key frequencies for the COO^- and the NH_3^+ groups in amino acids were discussed, and certain anomalies in the spectra of the amino acids (especially those containing sulfur atoms) were noted. Dr. Sutherland pointed out that, in the infrared spectra of synthetic polypeptides made from one or two amino acids, key frequencies are sometimes available for the identification of certain hydrocarbon groups in the amino acid residues. Recent work on the spectra of proteins using polarized infrared radiation to determine the orientation of NH and CO bonds was critically reviewed.

R. S. Halford (Columbia University) presented a careful and instructive discussion of the possible utility of infrared polarization data in assisting in the determination of the unit cells of certain organic compounds. He described his work with ammonium nitrate, and pointed out the various pitfalls that exist if inadequate crystallographic precautions are taken in the preparation of the samples. Dr. Halford emphasized the importance in infrred polarization studies of working with crystals cut along at least one of the major crystal axes. He then presented data showing infrared polarization obtainable with several types of oriented protein fibers.

The infrared spectra of nucleic acids, nucleosides, nucleotides, and certain purine and pyrimidine components were presented and discussed by E. R. Blout (Polaroid Corporation and Children's Medical Center). It was shown that certain of the infrared absorption bands of adenine and guanine could be used for analyzing mixtures of these two compounds in the solid state. A considerable discussion was evoked by the statement that oriented films of sodium desoxyribonucleate showed no apparent infrared dichroism in any of its absorption bands. Infrared polarization data for single crystals of cytosine and thymine were presented, but the data were not interpreted other than to point out that practically each absorption band of these compounds is highly polarized. It is evident that further fundamental work must be done in the field of infrared polarization of simple organic compounds before adequate theoretical interpretations can be made from the data that are beginning to accumulate in many laboratories.

The day's discussions were concluded with a presentation by Dr. Barer in which he emphasized some of the difficulties inherent in infrared microspectroscopic studies on tissues. In spite of the fact that resolution is low because of the long wavelength radiation used, it is hoped that interesting data will be obtained from tissues containing relatively homogeneous groups

of cells or, in certain special cases, single cells.

A lively symposium on the physical-chemical investigation of desoxypentose nucleic acid revealed many areas of agreement from different types of measurements, but emphasized the extraordinary difficulty of the molecular characterization of nucleic acids and the need for more definitive work.

The behavior of nucleic acids as polymeric electrolytes was discussed by D. O. Jordan (Nottingham). The nucleic acid polymer contains both strong and weak electrolytic groups, and the presence of the former and the ionization of the latter affect the viscosity of its solutions and the shape of the titration curve. The viscosity of desoxypentose nucleic acid shows considerable structural character, and the viscosity at low pressures is high. The addition of electrolyte reduces the viscosity considerably, as has also been described for simpler systems—for example, polymethacrylic acid and poly-*N*-*n*-butyl-4-vinyl pyridonium bromide. This process is explained by the repulsion of the charged groups (PO^- at pH 7) maintaining a stretched polymer molecule in aqueous solution. The addition of electrolyte reduces this repulsion, thereby permitting the molecule to coil, thus making it more symmetrical and reducing the viscosity, the structural character of the viscosity, and the streaming birefringence. In the absence of added salt, the reduced specific viscosity does not rise at low concentrations as that of a typical polyelectrolyte should. This is probably because of the incompleteness of removal of foreign ions from solutions of sodium thymonucleate.

The same sample of nucleic acid that had been used by Drs. Jordan and Ogston had been examined by light-scattering techniques by B. Bunce and P. M. Doty (Harvard). These measurements, which extended down to concentrations of 0.005 per cent, indicated a molecular weight of 4 million and a moderately coiled structure, the chain stiffness being comparable to that of cellulose derivatives. When the salt concentration is diminished below 0.2 *M*, uncoiling of the chain is noted.

The problems involved in the determination of the molecular weight of nucleic acids by means of the ultracentrifuge were considered by A. G. Ogston (Oxford). Marked molecular interaction makes it necessary to extrapolate the sedimentation and diffusion constants to zero concentration. The sedimentation constant S may be satisfactorily extrapolated as $1/S$ against concentration to give a limiting value for the desoxypentose nucleic acid of calf thymus of 13.2×10^{-13}. The extrapolation of the diffusion constant is performed with the aid of Flory's theory of the entropy of solutions of large particles to give a value of 0.80×10^{-7}. The molecular weight was then found to be 890,000, and the axial ratio is 120, which is to be compared with the value of 190 obtained from viscosity measurements when a high rate of shear is used. A possible reservation is made with respect to the diffusion measurements, since the concentration dependence is not certain. A more likely explanation of the disagreement, however, may be found in the possible heterogeneity of the nucleic acid preparation, since the light-scattering method gives a higher average value than the sedimentation and diffusion data. Evidence for the polydispersity was presented by K. G. Stern (Brooklyn Polytechnic) in the marked spreading of the sedimentating boundary at low concentrations. His sedimentation measurements on a Signer preparation in 0.2 *M* NaCl were in very good agreement with those of Dr. Ogston on a Gulland-Jordan preparation. In the absence of added salt the concentration dependence is so marked that extrapolation to zero concentration is not possible. H. Kahler (USPHS, Bethesda) reviewed his work on a nucleic acid preparation of Greenstein. Sedimentation and diffusion measurements led to a molecular weight of 1.5 million. M. Goldstein (Brooklyn Polytechnic) reported on preliminary streaming birefringence measurements on desoxyribose nucleic acid. The problem of concentration dependence of the rotary diffusion constant has not yet been solved, and here, as in the other kinetic methods of investigation, the interpretation of the data was seriously hampered by the necessity of relying on the prolate ellipsoid as the only model.

In separate papers Dr. Ogston described his recent work on the theoretical basis of extrapolating sedimentation constants to zero concentration, and J. L. Oncley (Harvard) summarized the work of his group of the β-lipoprotein of human sera. Dr. Ogston pointed out that, inasmuch as both sedimentation and diffusion constants of asymmetrical particles vary strongly with concentration, extrapolation to zero concentration is necessary if reliable values for molecular weights and shapes are to be obtained. In the case of sedimentation, only the frictional coefficient varies. This is not adequately accounted for by variation of the macroscopic viscosity. A theoretical treatment was outlined, based on the equations of flow through a porous plug, which predicts correctly the linear variations of $1/S$ with c. This may be used to estimate the effective volume and asymmetry of the particles. In the case of diffusion, the frictional coefficient may not be the same as in the sedimentation case and may be best measured by the macroscopic viscosity at zero velocity gradient. In addition, the diffusion coefficient varies with the entropy contribution to the chemical potential.

The β-lipoprotein discussed by Professor Oncley has

a density of only 1.03: it is spherical, having a diameter of 185 A and a molecular weight of 1,300,000. Only 23 per cent of the molecule is composed of polypeptide components, the remainder being phopholipid and cholesterol. In another paper Mr. Baldwin and J. W. Williams (Wisconsin) presented a general method for the determination of sedimentation constant distributions. The distribution completely defines the heterogeneity of the preparation with respect to sedimentation behavior, and the method is applicable even when the diffusion constant varies widely among the molecules. The theory does not apply to concentration-dependent systems, and all quantities must be extrapolated to infinite dilution. The standard deviation of the sedimentation constant distribution, as well as the weight average diffusion coefficient, can be calculated independently.

The organization of this conference was carried out by E. R. Blout and K. G. Stern. The success has led to plans for a similar conference during the last week in August 1951. This conference will be under the chairmanship of R. W. G. Wyckoff.

Technical Papers

The Effect of X-Irradiation in Oxygen and in Hydrogen at Normal and Positive Pressures on Chromosome Aberration Frequency in *Tradescantia* Microspores[1]

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Investigations by Giles and Riley (*1, 2*) demonstrated a marked effect of oxygen in increasing the frequency of x-ray-induced chromosomal aberrations in microspores of *Tradescantia paludosa*. In certain experiments (*2*), the relation between aberration frequency and percentage of oxygen present during irradiation was studied. Although there was an almost linear increase in aberration frequency between 2% and 21% oxygen (air), no significant difference was noted between 0% oxygen (pure helium) and 2% oxygen (plus 98% helium). Additional experiments have now been performed to reinvestigate the effect of low percentages of oxygen. In addition, studies have been made of the effect on aberration frequency of irradiation in various percentages of oxygen under pressure. Preliminary experiments have also been carried out on the effects of irradiation in hydrogen at normal and positive pressures. The general methods utilized for x-irradiation of inflorescences and subsequent cytological analysis have been described previously (*2*).

In the experiments designed to reinvestigate the effects of low percentages of oxygen, special attempts were made to remove as much oxygen as possible from the inflorescences before irradiation. The following procedure was utilized: (1) The inflorescences, after being placed in the gas-tight exposure chamber, were subjected to prolonged evacuation. (2) Helium, which had been especially freed of any residual oxygen, was then introduced into the evacuated exposure chamber. To remove any oxygen, the helium was passed slowly through a coil in liquid nitrogen and then over copper foil heated to 500° C before entering the exposure chamber. (3) Finally, the inflorescences were kept in the dark for an hour before irradiation to facilitate the removal by respiration of any further oxygen. In addition to the exposures in helium, irradiations were performed in atmospheres containing 2%, 5%, and 10% oxygen (plus helium). The percentages of oxygen in these gas mixtures were accurate (on the basis of analyses furnished by the vendor, the Ohio Chemical and Surgical Equipment Co.) to ±0.2%. All experiments were carried out at room temperature (approximately 25° C). To increase the statistical reliability of the determinations, a larger number of cells was scored than previously. The data are presented in Table 1. They are also plotted in Fig. 1, together with the averages of points at higher percentages of oxygen, which were obtained in previous experiments (*2*). These results indicate that there is still a substantial yield of aberrations even in the complete (or nearly complete) absence of oxygen. When oxygen is present during irradiation, there is a rapid rise in aberration frequency above this base level. This increase is linear between 0% and 10% oxygen, after which the rise is apparently more gradual, and shows a definite leveling off at around 20% oxygen.

TABLE 1

THE EFFECT OF IRRADIATION IN VARIOUS PERCENTAGES OF OXYGEN ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN *Tradescantia* MICROSPORES
(All X-Ray Exposures of 400 r at 50 r/min)

Oxygen percentage	No. cells	No. interchanges	Interchanges /cell	Interstitial deletions	I.D./cell
0	1,000	234	0.234 ± 0.015	287	0.287 ± 0.017
2	1,200	347	0.289 ± 0.016	394	0.328 ± 0.017
5	1,000	427	0.427 ± 0.021	501	0.501 ± 0.022
10	600	364	0.607 ± 0.032	453	0.755 ± 0.035

[1] Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.

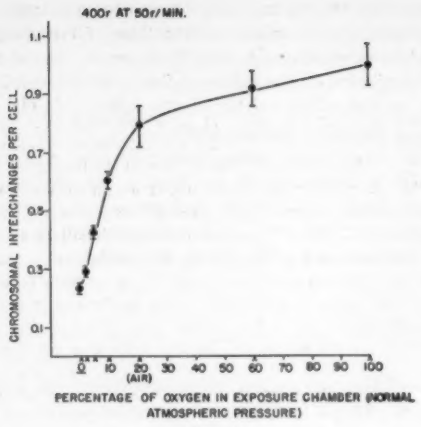

FIG. 1.

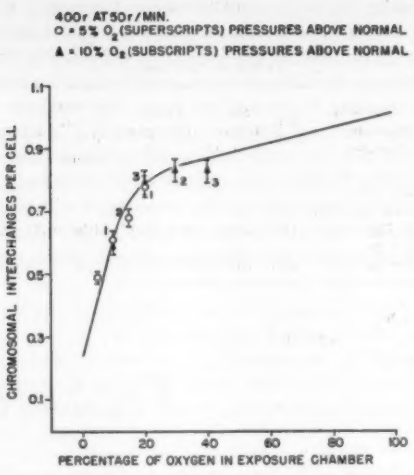

FIG. 2.

The irradiation experiments in atmospheres of increasing oxygen concentration just outlined were all performed at normal atmospheric pressure (*ca.* 740 mm of Hg at Oak Ridge). Since the amount of dissolved oxygen is directly proportional to pressure, an experiment was designed to test for an oxygen effect in another way—by carrying out exposures in which different sets of inflorescences were irradiated in the same oxygen-helium gas mixtures, but at pressures of 1, 2, and 3 atm above normal pressure. The same exposure chamber was used as previously, and the pressure inside the chamber was measured by means of a standard gauge on the gas cylinder, the accuracy of which was checked against a mercury manometer over the range of the first 2 atm above normal. The data for exposures in 5% and 10% oxygen (plus helium) are presented in Table 2, together with results obtained in air and in pure (*ca.* 99.8%) helium. The values for two separate experiments in 5% oxygen at normal pressure have been averaged in the table. During the exposure in compressed air at 3 atm above normal a small leak developed in the exposure chamber, and although the pressure gauge did not drop, it is felt that the value for this exposure may not be as reliable as for the other observations.

These results show that there is a marked increase in aberration frequency accompanying increasing pressure in those experiments in which oxygen was present during irradiation. When exposures occurred in the absence of oxygen (in pure helium), however, no increase resulted, indicating that pressure alone, at least within this range, did not influence the yield of aberrations. In Fig. 2 the

TABLE 2

THE EFFECT OF PRESSURE ON THE FREQUENCY OF X-RAY-INDUCED CHROMOSOMAL ABERRATIONS IN *Tradescantia* MICROSPORES IRRADIATED IN ATMOSPHERES CONTAINING VARIOUS PERCENTAGES OF OXYGEN.
(All Exposures of 400 r at 50 r/min)

Pressure in exposure chamber	No. cells	No. interchanges	Interchanges /cell	No. interstitial deletions	Interstitial deletions /cell
Irradiated in 10% oxygen (plus 90% helium)					
Normal atmospheric (*ca.* 740 mm Hg)	800	474	0.59 ± 0.027	599	0.75 ± 0.031
One atm above normal	550	436	0.79 ± 0.038	563	1.02 ± 0.043
Two atm above normal	700	584	0.83 ± 0.035	875	1.25 ± 0.042
Three atm above normal	700	584	0.83 ± 0.035	732	1.05 ± 0.039
Irradiated in pure helium (—99.8%)					
Normal atmospheric (*ca.* 740 mm Hg)	950	220	0.24 ± 0.016	256	0.27 ± 0.017
Three atm above normal	1,000	223	0.22 ± 0.015	259	0.26 ± 0.016
Irradiated in air					
Normal atmospheric (*ca.* 740 mm Hg)	1,000	713	0.71 ± 0.027	780	0.78 ± 0.028
Three atm above normal	950	786	0.83 ± 0.030	904	1.05 ± 0.033
Irradiated in 5% oxygen (plus 95% helium)					
Normal atmospheric (*ca.* 740 mm Hg)	1,850	903	0.49 ± 0.016	954	0.52 ± 0.017
One atm above normal	950	583	0.61 ± 0.025	671	0.71 ± 0.027
Two atm above normal	1,000	683	0.68 ± 0.026	802	0.80 ± 0.028
Three atm above normal	840	657	0.78 ± 0.031	783	0.93 ± 0.033

TABLE 3

THE EFFECT OF IRRADIATION IN HYDROGEN ON CHROMOSOME ABERRATION FREQUENCY IN *Tradescantia* MICROSPORES (400 r at 50 r/min)

Pressure in exposure chamber	No. cells	No. interchanges	Interchanges /cell	Interstitial deletions	I.D./cell
Normal atmospheric (ca. 740 mm Hg)	707	149	0.21 ± 0.017	166	0.23 ± 0.018
Three atm above normal	498	100	0.20 ± 0.020	124	0.25 ± 0.022

frequencies of chromosomal interchanges at various pressures in 5% and 10% oxygen have been plotted against the standard curve from Fig. 1, on the assumption that the amount of dissolved oxygen in the microspores is directly proportional to the pressure, and that this value determines the aberration yield at the standard dose of 400 r at 50 r/min. As can be seen from the graph, the agreement with previous results at comparable partial pressures of oxygen is very good. These results thus support earlier views that the amount of dissolved oxygen present in cells is an important factor in determining aberration frequency. They indicate that pressure alone, at least within the range up to 3 atm above normal, does not influence this type of radiation-induced change.

Several different types of evidence (*3*) support the view of Thoday and Read (*4*) that the effect of oxygen in increasing chromosome aberration frequency may result from an indirect action of x-radiation to decompose water with the production of hydrogen peroxide. However, although very little hydrogen peroxide appears to be produced by x-rays in oxygen-free water (*5*, *6*), it will be recalled (Table 1) that there is still a substantial aberration frequency in inflorescences exposed in purified helium to 400 r. The question naturally arises as to the mechanism by which these aberrations are produced. It seems quite probable (*3*) that the effect of a substance such as hydrogen peroxide on aberration frequency would arise from an increased production of chromosome breaks, rather than from an influence on the reunion behavior of broken ends. If this is the case, aberrations induced by x-rays in the absence of oxygen might result (at least in part) from breaks produced by another substance, the OH radical, which is formed by the radiodecomposition of oxygen-free water. On the other hand, all the aberrations produced in the absence of oxygen might arise from the *direct* action of the radiation in ionizing the molecules of the chromosomes themselves.

A preliminary attempt has been made to distinguish between these two possibilities by means of experiments designed to remove the OH radical by promoting, during x-irradiation, the back reaction to form water. Allen (*6*) has shown that the presence of molecular hydrogen markedly accelerates this back reaction. Consequently, inflorescences were irradiated in pure hydrogen at normal atmospheric pressure and at 3 atm above normal. These data are presented in Table 3. Although there is some decrease in aberration frequency compared to earlier results obtained with irradiation in helium, the differences for the most reliable observations, those of interchanges, are not significant. In this experiment it was not possible to obtain evidence that hydrogen was actually present in the cells during irradiation. However, previous experiments with oxygen (*2*) have indicated that under similar conditions this gas diffuses into microspores very rapidly. Thus, despite the fact that hydrogen is less soluble in water than is oxygen, it seems reasonable to conclude that a considerable amount of hydrogen would be present in the microspores during irradiation, and that this should react to remove the OH radical. Consequently, the failure to find a significant decrease in aberration frequency when x-radiation is performed in hydrogen may mean that all the residual aberrations induced by x-rays in the absence of oxygen arise as a result of a *direct* effect of the radiation on the chromosomes. It should be pointed out, however, that this conclusion is based on the assumption that reactions leading to H_2O_2 production or suppression in cells from which oxygen has been removed as completely as possible are similar to those occurring in oxygen-free pure water. There is as yet little experimental evidence on this point, and it is quite possible that the complexity of the cellular environment may cause very different reactions to occur.

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The Detection of Internally-borne Bacterial Pathogens of Beans by a Rapid Phage-Plaque Count Technique[1]

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The use of specific bacteriophages for identifying bacterial species or strains is a valuable aid in medical and epidemiological work; phage typing of enteric pathogens, staphylococci, and streptococci is extensively employed (*1*). Phage has also been widely used to separate closely related groups, species, and strains of bacteria for purposes of classification (*1*, *2*). Within recent years bacterial viruses have been recognized as very useful tools in the rapid identification of plant

[1] Contribution No. 302.

[2] The author gratefully acknowledges the cooperation of M. D. Sutton, Division of Botany and Plant Pathology, in preparing the bean samples and carrying out the pathogenicity tests

pathogenic bacteria (*3, 4*); organisms distinguishable only by pathogenicity may be readily separated by specific phages (*5*).

No satisfactory rapid method is available for detecting internal infection of seed by phytopathogenic bacteria such as *Pseudomonas phaseolicola* and *Xanthomonas phaseoli*, which cause halo and common blight, respectively, of beans. Serological techniques may hold some promise, but plant pathological laboratories are not generally equipped to use them. In the present work typical phages were isolated for these two organisms from a mixture of soil, compost, and sewage and were found to lyse all strains of these species tested but no other species in the same genera. The phage for *X. phaseoli*, for example, did not lyse *X. phaseoli* var. *fuscans* or *X. phaseoli* var. *sojensis*. Such specificity has also been reported by Thornberry *et al.* (*4, 5*) working with *X. pruni* phage. The above phages were used successfully in identifying isolates from suspected bean samples.

If the pathogen were present in a macerated bean sample in any number, and if a known number of phage particles were added to a given volume of this sample, a significant increase in number of phage particles should occur after a suitable interval, if conditions are favorable and contaminating organisms and the bean tissue itself do not interfere. Such conditions will eliminate the need for plating, isolating, and phage-testing. This theory was based on the well-established fact that one susceptible bacterial cell infected with one phage particle may liberate as many as 20–100 or more particles ("burst size"), depending on the bacterial species and the conditions of growth (*6*). Experiments along these lines gave results that supported the above hypothesis.

TABLE 1

PRESENCE OF *Pseudomonas phaseolicola* IN BEANS AS INDICATED BY MULTIPLICATION OF SPECIFIC PHAGE ADDED TO MACERATED TISSUE

	Series	Plaque count/0.1 ml	
		When phage added	After 6-hr incubation
1.	Nutrient broth	30	35
2.	1. + cells of *P. phaseolicola*	25	Confluent plaques
3.	Sterile bean tissue	24	12
4.	3. + cells of *P. phaseolicola*	20	Confluent plaques
5.	Bean samples		
	RT 1	24	1,200
	RT 4	26	Confluent plaques
	RT 5	26	" "
	RT 8	25	1,150
	RT 10	40	1,450
	RT 13	22	3,200

The method finally evolved consists of surface-sterilizing 50 bean seeds (with chlorine), washing, and grinding for 5 min in 250 ml sterile nutrient broth in a Waring Blendor. The mixture is retained at room temperature overnight, after which it is shaken and 10 ml of the turbid supernatant removed aseptically into a sterile flask; 1 ml of a suitable dilution of phage containing a known number of virus particles (about 4,500 in most of our experiments) is then added to the flask. Plates are poured immediately with 0.1 ml of the mixture in the flask, 0.6 ml of a nutrient broth suspension of cells from a 24-hr slant culture of the homologous organism ("substrate cells"), and 10 ml yeast-beef agar. Plaques may be counted after 5–8 hr at room temperature. The contents of the flask are allowed to stand at room temperature for 5–6 hr, after which 0.1 ml or smaller amounts of the mixture (obtained by dilution) are again plated and plaques counted after 5–8 hr. Where the specific organisms are present in a sample, the number of plaques increases strikingly (Table 1), frequently producing one confluent lytic area over the entire plate—unless, of course, suitable dilutions are made. Plaques show up despite rapid growth of saprophytic bacteria, which frequently are present in the sample; these may be suppressed to some extent if necessary by increasing the volume of the cell suspension used when plates are poured or by means of a suitable antibiotic. The number of phage particles added to the original flask is arbitrary; a sufficient number should be used to permit ready contact with the susceptible cells in the sample, allowing for some nonspecific adsorption by constituents of the ground beans, but not so many as would necessitate time-consuming dilutions of the sample prior to plating. In the above procedure 0.1 ml of the phage-bean mixture gives about 20–40 plaques per plate at zero time and usually from 20–100 times that number after 5–6 hr if the host cells are present.

By this method 11 out of 14 samples of beans were shown to be infected with *P. phaseolicola*. The positive and negative samples were also plated out, colonies on representative sections of suitable plates picked, and the resulting cultures tested for phage lysis and for pathogenicity. To date only the isolates from positive samples, which were susceptible to lysis, were found to be pathogenic to bean seedlings.

The size of sample was found to be of great importance in these tests. This is of course conditioned by the degree of infection in the field: in a heavily infected sample 50 seeds are sufficient; in a lightly infected sample 200 or more may be required to give a positive result. Where 200 were required they were divided into 4 lots of 50 seeds each in 250 ml of broth before grinding.

Specificity of the virus is obviously of prime importance in this procedure, nor can the possible occurrence and development of resistant strains in the sample during the preliminary incubation be dismissed. However, the isolation of phage-susceptible pathogenic cultures from samples giving a positive reaction by the plaque-count technique and their absence in negative samples indicate that for this species at least, resistant strains are not a serious obstacle. The possible presence of a specific phage in the sample has also been considered, and in fact phage has been encountered several times. By pouring plates containing susceptible "substrate" cells immediately after grinding the beans and after the preliminary overnight incubation, such phages can be detected. If they have increased in amount significantly overnight they themselves may serve as indicators of in-

fection of the beans, provided their specificity is established.

Preliminary tests with beans infected with *X. phaseoli* have indicated that this method holds promise in the diagnosis of common blight as well. This procedure may be applicable to the rapid detection of both plant and animal pathogens or saprophytic organisms in various complex substrates or in simple mixtures of closely related forms.

Details of the experiments discussed above will be published elsewhere.

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The Potentiation of Muscular Contraction by the Nitrate-Ion[1]

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It has long been known that frog skeletal muscles that have been exposed to various anions of the lyotropic series yield potentiated contractions when stimulated by such agents as cold, potassium, or electric shock (*1–5*). There is general agreement in this earlier work that the ions cause a "sensitization" of the excitatory membranes of the muscle fibers, which is then effective in increasing the mechanical response of the whole muscle. Thus, in work most directly pertinent to our own research, Chao (*4*) studied the behavior of frog sartorii exposed to a Ringer's solution normal with respect to potassium and calcium chlorides, but having all the NaCl replaced by an equivalent amount, e.g., of $NaNO_3$. Such muscles were found to quickly develop a reduction in rheobase for electric stimulation; and, furthermore, if they were stimulated with twitch shocks of a *submaximal* intensity, as determined for these muscles when previously equilibrated to ordinary Ringer's solution, their isotonic contractions became greatly augmented after only a few minutes' contact with the nitrate medium. Chao attributed the mechanical potentiation to recruitment of fibers; i.e., the lowering in threshold of the fibers of the nitrate-treated muscle permitted response in those fibers that were not excited by the submaximal shock in normal Ringer's solution, and thus, by adding their outputs to the total twitch response, caused the observed potentiated contractions. In this investigation the effects of other anions were similarly tested, and it was found that the degree of lowering of the rheobase and of the associated enhancement of shortening by submaximal stimulation was a function of the particular anion, the relative effects falling into the usual lyotropic series, $Cl < Br < NO_3 < I < CNS$.

In our research, which is reported here in preliminary form, we have used a procedure rather similar to Chao's, with the important exception that stimulation was effected by originally slightly *supermaximal* shocks. Under these conditions, irrespective of the lowered threshold of the NO_3-treated muscle, no recruitment of fibers is possible; yet such muscles produce greatly potentiated twitches approaching, or even equalling, the strength characteristic of full tetanus output. Full analysis of our results proves that the nitrate ion not only results in an increase in excitability but also causes, among other things to be discussed below, augmentation of the maximal mechanical response of which each fiber is capable.

We have studied the isometric twitch responses of excised curarized[2] frog sartorii when supermaximally shocked by the massive, transverse stimulation procedure (*6*). Records have been made by piezoelectric, cathode-ray methods (*7*) of the latent period changes and, using optical myography, of the associated developed peak tension outputs. All experiments have been done at 25°C. After 1 hr of equilibration in oxygenated phosphate buffered (pH 7.2) normal Ringer's solution, the behavior of the muscle was recorded for 2 responses separated by an interval of 1 min. This chloride-Ringer's solution was then withdrawn from the muscle chamber and replaced by a similarly buffered nitrate-Ringer's solution, through which oxygen was bubbled. In our test medium *all* the chloride was replaced by nitrate, and it is in this sense that we shall refer to our experimental medium as a nitrate-Ringer's solution. In this new environment the muscle was tested at intervals, the stimuli being again massive, transverse electric shocks of the same supermaximal strength as those applied to the muscle in normal Ringer's.

The results of a representative experiment are presented in Fig. 1. The inset at the top of this figure is a diagrammatic sketch of a typical photographic record obtained with our apparatus. The curved line, corresponding to the deflection of the cathode-ray beam in a single sweep, gives the mechanical changes that appear during the twitch latent period. The arrow at the left indicates the instant of stimulation. Following this moment 2 time intervals are presented: L_R, which measures the duration of a mechanically quiescent period —i.e., the latency of the immediately following latency relaxation (LR); and L, which measures the time interval of the mechanical latent period for positive tension development. After the termination of L, the record shows in the upward deflection the very beginning of the contraction period. Also on this sketch are indicated 2 tension variables: R, measuring the depth of the LR;

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and the New York University (NR113-300).

[2] We are very grateful to E. R. Squibb & Sons for generously supplying us with the *d*-tubocurarine chloride used in these experiments.

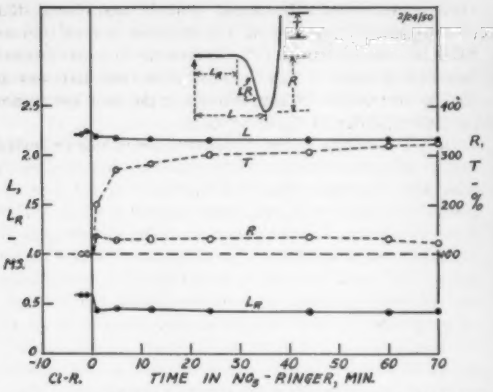

FIG. 1. Effect of nitrate-Ringer's solution on the latent period and peak-developed tension responses of the isometric twitch of the frog sartorius muscle.

and T (upper right corner), which measures the peak tension developed by the contraction. (The upper horizontal line represents the light beam of the optical myograph at a level representing the muscle's resting tension, and the lower line corresponds to the displaced position of the beam at peak of contraction.)

The solid graphed lines give the changes in L_R and L, with the time scaling indicated in ms on the left, vertical axis. Both these time intervals decrease by 0.1–0.2 ms immediately upon immersion of the muscle in the NO_3-Ringer's, and these decreases are maintained throughout the remainder of the 70-min exposure to the experimental medium. These are very small diminutions, but they are definite and have been repeatedly obtained in every experiment performed. The variations in R and T (dashed lines), with their scaling (right vertical axis) in per cent of the chloride-Ringer's outputs, also occur very soon after the muscle is put into the nitrate-Ringer's; the increase in R is about 50%, whereas T is maximally potentiated to about 325%—i.e., to a value about as large as the maximum tetanus tension. Thus, as compared to the chloride-Ringer's behavior, the muscle, when placed in the modified salt solution, quickly develops a small but certain speeding up of the latent period events, a considerable deepening of the latency relaxation, and a quite enormous increase in developed tension. Furthermore, although the data are not shown in the figure, the nitrate-induced changes are all rapidly reversed when the muscle is restored to a normal Ringer's bath.

The highly potentiated peak tensions of the nitrate-Ringer's muscles cannot be accounted for by recruitment of fibers, since the supermaximal shocks we used throughout ensured that even in the chloride-Ringer's all the available fibers of the muscle were stimulated. Nor can these potentiations be a consequence of a sort of tetanization that would occur if the single shock we used evoked a series of action potentials, as occurs, for example, in a veratrinized muscle. For a study of the electrical response of the muscle after exposure to the nitrate-Ringer's (using wire-electrode stimulation and conventional recording technique, with the muscle in a moist chamber) proves that only a single, evidently normal, action potential occurs. This result is shown in Fig. 2, which presents the action potential response of the chloride-Ringer's muscle in the upper record, and of the nitrate-Ringer's muscle in the lower. The much greater tension output of the experimental muscle is incidentally demonstrated also by comparing the tension beam deflections of the 2 muscles in the upper right portion of each record. Our results thus prove that the responses in the nitrate-Ringer's solution are potentiated maximal twitches in which each fiber produces a peak tension of the order of its maximal tetanus strength.

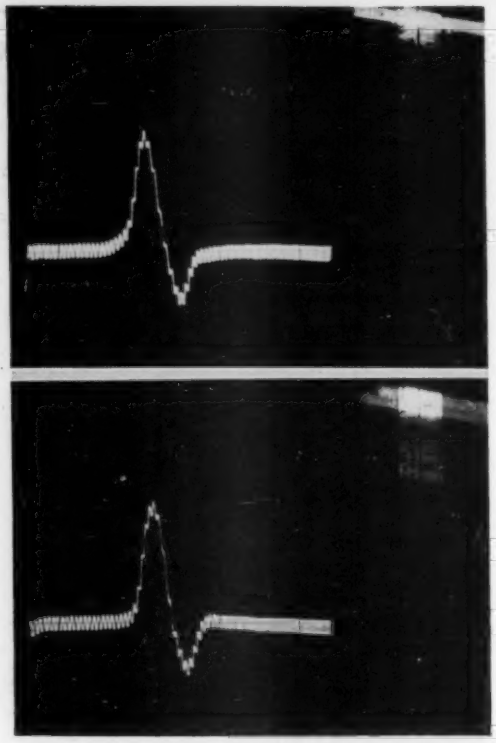

FIG. 2. Records of action-potential and peak-developed tension responses in isometric twitch of frog sartorius muscle: in chloride-Ringer's (upper photograph), and in nitrate-Ringer's (lower photograph). A 5,000 c/sec timing wave is superimposed on each action potential record.

Following Chao's procedure, we have also studied the effects of submaximal shocks (50% of maximal), as determined in chloride-Ringer's, on muscular responses in the nitrate-Ringer's. Here, again, all electrical responses consist of a single action potential, which, however, is much larger in the nitrate medium, thus indicating recruitment of fibers made possible by the nitrate-induced increase in excitability. But the mechanical response of these muscles is very much larger than would be expected if only recruitment of mechanically normal fibers were

involved. Hence, our results in general prove that the nitrate ion does not merely reduce the threshold, but—and this is the essential new finding of our research—it also can somehow modify the events of the latent period and increase the contractile strength of each excited fiber.

It is significant that the effect of nitrate on the mechanical behavior, both of the latent period and of the peak tension development of the contraction period, occurs so rapidly after immersion of the muscle in the nitrate-Ringer's solution. This indicates that the nitrate ion could not have had sufficient time to penetrate into the muscle fibers and thus modify the mechanical response by a direct action on the contractile system. We therefore infer that this ion directly affects only the surface—i.e., the excitatory membrane of the fibers—and that this alteration leads, in turn, to the observed changes in the mechanical response by a modification of the mechanism by which excitation of the membrane by the electric shock is coupled to the inner contractile system.

That the membrane is altered is clear. This is proved by the increase in excitability. But it is also demonstrated by the fact that the nitrate ion slightly increases the resting potential (*5*) and considerably enhances the permeability (*8*) of muscle cells. Probably, as Chao suggests (*2, 3*), the increased excitability is a consequence of the greater dispersibility of the membrane under the influence of nitrate, and the possibility that this structural change may underlie the other differences in membrane properties we have mentioned is not excluded.

Our principal interest, however, lies in the influence of nitrate on the mechanism of excitation-contraction (*E-C*) coupling. Our results indicate that this ion has its initial effect on the excitation part of this process. This influence, however, is not on the reactions responsible for the action potential, since the electrical response of the excitatory membrane of each fiber is unaffected. It would therefore seem that some other feature of the excited membrane is altered by the nitrate ion, and that this occurs in such a fashion as to change the subsequent course of the coupling mechanism. The time course of *E-C* coupling extends from the instant of stimulation to the onset of tension change—i.e., it is coincident with the events of the latent period, or at least with the earlier phases of this interval. Hence, it is not surprising that under the influence of nitrate we record latency alterations—the shortening of L_R and L, and the deepening of the latency relaxation. Previous work (*7*) has shown that the LR is an expression of the contractile material undergoing activation for its positive tension development. It is therefore evident that these latency mechanical changes may reflect the modified course of the latter portion of *E-C* coupling, during which the chain of events initiated by the action of the stimulus on the membrane becomes linked to the contractile material, and in such manner, enables the fiber-tension output to be so greatly potentiated.

Needless to say, the full elucidation of the mechanisms discussed above requires the performance of much more research. We plan to study the effects of other anions and of a variety of other agents (e.g., of the cholinesterase system) that are believed to play a role in, or to affect, excitability mechanisms. Some insight into the complexity of the processes is indicated in already reported results (*9, 10*) concerned with the effects of the potassium ion. This agent in relatively small concentrations acting for prolonged periods, or in larger concentrations acting for short durations, increases R up to 300% or more, potentiates T about 25%—effects that are irreversible—and reversibly depresses the action potential. Here, as in the NO_3 effects, the various changes are due to the direct action of K on the excitatory membrane. Yet, as can be seen by comparing the noted actions of these two ions, considerable differences exist regarding the quantitative alterations in R and T, the action potential behavior, and the possibility of reversal of the induced changes. It is felt, however, that a systematic study of these phenomena will shed some light on the fundamental mechanisms of excitation-contraction coupling, and, since this process activates contraction, it is hoped that any knowledge gained concerning it may also help in furthering comprehension of the contraction process itself.

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The Mechanism of Action of Organic Mercury Compounds on Cytochrome Oxidase

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Several organic mercury compounds (phenylmercuric nitrate [PMN], phenylmercuric hydroxide [PMOH], and *p*-chloromercuribenzoic acid [benzoate]), known to react with the –SH group, have been shown to inhibit the cytochrome c–cytochrome oxidase system at the oxidase portion of the chain (*1*). Since Barron (*2*) and others have considered this oxidase not to be sulfhydryl-dependent, it was suggested that the inhibition of the oxidase (a crude rat heart preparation) was nonspecific and might be due to the particulate nature of the preparation.

Investigations presented in this note, in which a solubilized sodium desoxycholate preparation of cytochrome oxidase (*3*)[1] was used, show that this partially purified enzyme is also inhibited by the mercurials. The in-

[1] The cytochrome oxidase was generously supplied by S. J. Cooperstein, of Western Reserve University.

hibition measured manometrically[2] was of the same degree as with the crude enzyme. When rates of oxidation of reduced cytochrome c by the treated oxidase were determined spectrophotometrically, the inhibition was less than with the crude enzyme (Table 1). This decrease in inhibition was especially marked with the benzoate as inhibitor; higher concentrations of this reagent (oxidase: inhibitor ratio 1:3.04) caused a slight (20%) depression in the rate of oxidation.

TABLE 1

Mg ratios* Oxidase : Inhibitor	Average % depression			
	Manometer		Spectrophotometer	
	Crude	Purified	Crude	Purified
1 : 1.80 PMN	100	100		
1 : 0.04 PMN	50	52.5	51.0	40.6
1 : 1.26 PMOH	100	100		
1 : 0.04 PMOH	50	56.4	46.5	34.9
1 : 2.10 Benzoate	100	100		
1 : 0.16 "	50	44.2	67.3	0

* In terms of crude enzyme. Purified enzyme was approximately 5 times as active, and corresponding adjustment in oxidase : inhibitor ratio was made.

The mechanism of action of the mercurials was studied by means of spectral analysis. After treating the oxidase with the mercurials in mg ratios of oxidase to mercurials which gave nearly 100% depression manometrically, the shifts in the visible spectral bands shown in Table 2 were observed. In concentrations of mercurial giving only 50% depression of the oxidase, the shift in the reduced bands was not as great (cf. visible spectra in [4]).

TABLE 2

	Oxidized bands mμ	Reduced bands mμ	
Untreated enzyme	417–419	443	601–603
Treated "	415–417	433–435	593–599

The effect of the mercurials on the ultraviolet spectrum is to increase the extinction throughout the region examined, 235–320 mμ (Fig. 1). The observed maximum at 277–278 mμ and minimum at 250–52 mμ were not shifted significantly, nor did any new bands appear. The extinction was particularly increased over the bands and showed least rise in the region between 285–300 mμ. In mixtures containing higher concentrations of –SH reagents (those giving nearly 100% depression), a clouding of the solution, which could not be removed by centrifugation or Seitz filtration, caused a high nonspecific absorption which masked both the maximum and minimum (Curve 4, Fig. 1). No clouding was visible macroscopically when concentrations of mercurial giving 50% depression were used. However, the rise in extinction which was also observed in these experiments indicates that an increase in density did occur.

[2] See reference (1) for manometric and spectrophotometric procedures.

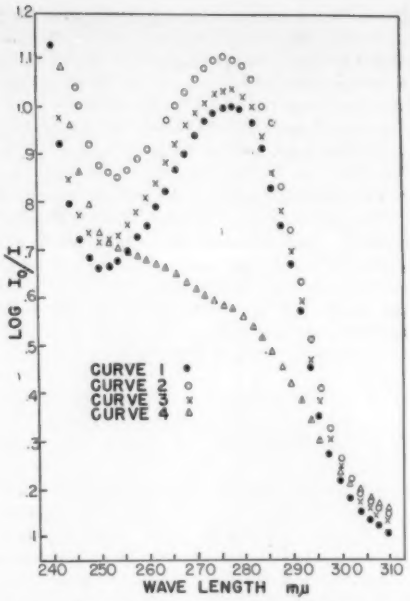

FIG. 1. Effect of chemical and physical treatment on the ultraviolet spectrum of cytochrome oxidase. Curve 1, control; Curve 2, oxidase-PMOH in mg ratio of 1 : 0.05; Curve 3, heat-denatured oxidase, 56° C for 15 min; Curve 4, oxidase-PMN in mg ratio of 1 : 0.5, solution diluted in 1 : 2. Concentration of oxidase in Curves 1, 2, and 3 is 10 mg/ml; in Curve 4, 3.3 mg/ml.

Study of chemical inactivation by the mercurials was paralleled by a similar study of physical inactivation by heat. The rate of oxidation of cytochrome c by oxidase heated for various lengths of time at 56° C was measured. Under the conditions of our experiments, heating for 30 min at 56° C caused complete inactivation of the enzyme. No macroscopically visible clouding occurred.

Spectral studies on this heat-denatured enzyme showed the same type of increase in extinction in the ultraviolet, although the percentage of increase was considerably less than that caused by the mercurials. Likewise, the same shifts were observed in the reduced spectrum in the visible region. In the oxidized spectrum no shift occurred.

Although the effect of the mercurials is more drastic, the type of inactivation in the two instances appears to be similar—a denaturation of the protein moiety. As a result of the alteration of its protein component, the bands in the visible region characteristic of the prosthetic group in the intact enzyme undergo a shift, and the specific absorption attributed to the native molecule is greatly decreased with an increase in nonspecific absorption.

Phenylmercuric hydroxide and, especially, *p*-chloromercuribenzoate have been employed as specific reagents for the sulfhydryl group in the protein molecule (5). The present studies show that in concentrations commonly used for this purpose these reagents can inactivate an

enzyme generally believed to require no –SH group for its function. Slater (*6*) has also noted that *p*-chloromercuribenzoate "has some effect on cytochrome oxidase." It has been shown that phenylmercuric nitrate can inactivate other nonsulfhydryl enzymes (*7*). It should be emphasized that, although it is possible to protect cytochrome oxidase against mercurial inhibition by means of cysteine or other sulfhydryl compounds, it is not possible to reverse the inhibition by such means (*1, 8*). When enzyme inhibition by mercurials is due to combination with essential –SH groups, such reversal can normally be accomplished (*5*).

Details of these investigations and additional data concerning the mechanism of inactivation will be published elsewhere.

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Differentiation of Bacterial Species and Variation within Species by Means of 2,3,5-Triphenyltetrazolium Chloride in Culture Medium[1]

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When different species or varieties of bacteria are grown as separate colonies on a clear agar medium, such as tryptose agar, containing 0.01% or less of triphenyltetrazolium chloride, the colonies of each will develop either a different shade of red coloring or a similar shade of red along with a different pastel color in the borders of the colonies. The latter colors, often the most pronounced differences seen, are tints of green, blue, yellow, or red.

The following procedures are recommended for use in the detection of colonial differences in bacteria: A 1% solution of tetrazolium chloride is prepared in distilled water and sterilized at 115° C for 15 min. One ml of this solution is added to 100 ml of melted tryptose agar and mixed well just before pouring on Petri plates. The depth of the agar in the plates should be approximately 0.5 cm. Since a solution of the salt is colorless in the oxidized form, the agar, after pouring, will remain colorless. The poured plates should be incubated at 37° C for about 20 hr to evaporate the excess moisture from their surfaces.

[1] Journal article No. 1129 n.s., Michigan Agricultural Experiment Station.

The surface of the agar plate should be inoculated in such a way that the colony population that arises will not be crowded. The bacterial suspension should be prepared in sufficient dilution so that when 1 cm is spread over the surface of the agar, from 200 to 400 colonies will arise on incubation. The period of incubation at 37° C before examination for differences in color will depend upon the rapidity of growth of the microorganisms under study. Those falling in the genera *Salmonella, Shigella, Escherichia, Alcaligenes,* and *Streptococus* should be incubated for 48 hr. Agar plates inoculated with *Brucella* should be incubated for 4 days. For the differentiation of *Staphylococci* and certain aerobic spore-bearing *Bacilli,* the concentration of the salt in the agar medium should be reduced to 0.0025%, as higher concentrations inhibit their growth.

The color patterns and differences in color patterns of bacterial colonies can best be seen by examining the growth on agar Petri plates under a low power (×12) dissecting microscope, illuminated by reflected, oblique light from the new Spencer microscope lamp. The light is focused at an angle of 40° on a small concave mirror placed in front of the microscope. The rays are reflected at an oblique angle from the mirror and strike the under surface of the Petri plate resting on the glass stage.

Thus far 15 different species or varieties of bacteria of 7 different genera have been studied. The bacterial cultures examined were from a culture collection. Each had been previously identified as to its genus, species, and variety by standard laboratory methods. The colonial variants of the species of *Brucella* used were from a large collection previously separated and identified by the senior author. With the exception of *Brucella,* no previous attempt had been made to bring about dissociation of the cultures studied.

All the varieties of *Salmonella* cultures examined, with the exception of *S. typhimurium,* showed two or more easily distinguishable colonial growth phases. Each phase could be distinguished from the other by differences in size, shape, the shade of red in the central area, and pronounced differences in delicate pastel tints in the border. The culture of *S. typhimurium,* showed only one growth phase. The color of the colonies was a light shade of red extending from the center to the border. Each of two cultures of *E. coli* showed two growth phases, differing in shades of red in the central area and pastel tints in the borders of the colonies. One culture of *S. sonnei* showed two distinct colonial growth phases, one of which was larger and more irregular in shape than the other. The larger colonies contained irregular deposits of deep red color superimposed on a lighter shade of red; the border was tinted light yellow. The smaller colony was more circular; the central area was colored light red and the border a light yellow-green.

Colonies of the smooth phase and closely related phases of the 3 *Brucella* species can be distinguished from each other more easily on agar medium containing tetrazolium chloride than on regular tryptose agar. Colonies of the 3 species and all growth phases of each are circular in shape. The central circular area of S phase colonies of *Br. suis* is stained a darker (almost black) shade of red

than that of *Br. abortus*. The same area in colonies of *Br. melitensis* is stained a lighter shade of red than that of *Br. abortus*. Another marked difference is seen in the circular borders of the colonies. The borders of *Br. suis* colonies are colored a light opaque yellow; those of *Br. abortus* a light, almost transparent bluish green; and those of *Br. melitensis* a light opaque pink.

The color differences in colonies of various species of bacteria and in their colonial variants do not change rapidly after the maximum color differences first develop. The most marked change that occurs with age takes place in the colony borders, which become more opaque. Color differences exhibited by colonies of distinct species of bacteria or their different phases of growth on a medium containing tetrazolium chloride are due to variations in the ability of their enzyme systems to reduce the compound to insoluble red triphenyl formazan (*1*).

The results obtained thus far by this method indicate that the procedure can be used as a means of identifying bacterial species or variations that occur within a species. It is highly useful as a means of detecting colonial variations in a supposedly pure growth phase culture of bacteria.

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Obligate Autotrophy in *Chlamydomonas Moewusii* Gerloff

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In a recent issue of Science, Finkle, Appleman, and Fleischer (*1*) reported that they have been unable to grow a certain strain of *Chlorella vulgaris* in the dark. Although many species of algae, including other strains of *Chl. vulgaris*, can be grown readily in the dark on simple organic substrates, other very closely related species apparently cannot be grown at all under these conditions. This presents a fascinating problem in the investigation of autotrophic and heterotrophic nutrition of algae—e.g., the species of *Euglena* studied by Dusi (*2*).

Some species of *Chlamydomonas* may be grown heterotrophically. Klebs (*3*) was able to grow *Chl. media* in darkness on sucrose media. Out of 7 species investigated by Lucksch (*4*), 6 (*Chl. dorsoventralis, monoica, pulchra, pseudagloë, pseudococcum*, and *subglobosa*) could be grown in darkness on media containing various sugars or acetate. *Chl. humicola* appeared to require light for growth, although carbon dioxide was not essential. It was deduced, from experiments with various algae, including *Chl. pseudococcum*, that light exerted some beneficial effect other than its action on photosynthesis (*5*). Jacobsen (*6*) did not obtain dark growth with 3 species of *Chlamydomonas* (*Chl. Ehrenbergii, intermedia, variabilis*).

Gerloff (*7*), in his description of *Chlamydomonas Moewusii*, stated that cells of this species are unable to multiply in darkness. Experiments to overcome the "dark block" in *Chl. Moewusii* (strains isolated by L. Provasoli in 1948) have been carried out here during the past year. No cell multiplication has been obtained in mineral media containing, or enriched with, any of the following organic compounds. (At the concentrations used, the growth of controls in the light was not inhibited.) All media were adjusted to pH 7. Sterilization was by autoclaving or by Seitz filtration.

Acids	*Hexoses*	*Phosphate Compounds*
Acetate	Glucose	Glucose-1-phosphate
Citrate	Galactose	ATP (animal)
Isocitrate	Fructose	Glycerophosphate
Fumarate	Mannose	Phosphoglycerate
α-Keto-glutarate		Adenylic acid
Lactate	*Pentoses*	Creatine
Malate	L-Arabinose	
Malonate	D-Arabinose	*Nitrogen Compounds*
Oxalo-acetate	Rhamnose	Alanine
Pyruvate	Ribose	Asparagine
Succinate	Xylose	Glutamine
Oxalate	Sorbose	Urea
Formate		
Thioglycolate	*Disaccharides*	*Various*
Propionate	Sucrose	Soluble starch
Valerate	Trehalose	α-Methyl glucoside
Butyrate	Maltose	Peptone
	Lactose	Tryptone
Alcohols	Melibiose	Coconut milk
Ethanol	Cellobiose	Yeast extract
Butanol		Liver extract
Glycol	*Trisaccharides*	Casein hydrolysate
Glycerol	Raffinose	Synthetic vitamin
Mannitol	Melezitose	mixture
Dulcitol		Gelatin
Sorbitol		Ascorbic acid
		Thiamine

A 5% carbon dioxide-air mixture was found equally ineffective when used supplementary to many of these carbon sources. Cell extracts, hydrolysates, and filtrates of light-grown cells do not support growth of cells in darkness; there seems to be no photostable toxic substance, produced by cells stored in the dark, inhibitory to cells growing in the light. Since *Chl. Moewusii* can perform the oxyhydrogen reaction in the dark (*8*), it was thought that energy derived from such a reaction might be utilized for growth. In other words, a chemoautotrophic mechanism might replace photosynthesis. However, an attempt to obtain growth in the dark in the presence of a gas mixture of 5% air, 5% carbon dioxide, and 90% hydrogen was unsuccessful.

It seemed possible that the cell membrane might constitute a barrier preventing the entry of organic substrates that could support growth. The problem, however, is apparently not one of permeability, since Warburg manometric experiments have shown that compounds such as acetate, pyruvate, or succinate enter the cells and are readily oxidized in the dark. Energy released by oxidation of these substrates does not appear to be available for the multiplication of the organism. Possibly some energy-transfer mechanism is lacking.

In the absence of carbon dioxide, no growth has been observed on any substrates in light or darkness. Until further experiments prove otherwise, *Chl. Moewusii* must

be considered an obligate autotroph, absolutely requiring both light and carbon dioxide for growth. This is analogous to the situation in *Thiobacillus thiooxidans* (*9*), where sulfur and carbon dioxide are required for growth and can be replaced by no other energy or carbon source, respectively.

Possibly some simple compound that we have overlooked will suffice to support growth in darkness. It may be, however, that for the growth of some organisms, such as *Chl. Moewusii* and *Th. thiooxidans*, one type of energy source is not replaceable by another. Herein lies the problem of obligate autotrophy.

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A Coenzyme of Spleen β-Glucuronidase[1]

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The preparation of an electrophoretically homogeneous β-glucuronidase from calf spleen has been described recently (*1*). The activation of β-glucuronidase by nucleic acids (*2*) and the existence of naturally-occurring inhibitors of β-glucuronidase (*3*) have been pointed out.

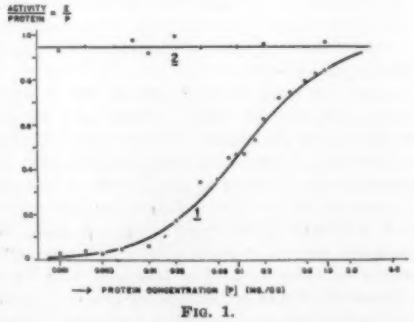

Fig. 1.

It has now been found that the activity of pure spleen β-glucuronidase is not proportional to the enzyme concentration, in contrast to the behavior of less pure preparations. The ratio of enzyme activity to protein concentration (E/P) drops greatly with dilution. Our experimental activity values (circles, Fig. 1) with increasing dilution coincide with a theoretical curve (Curve 1, Fig. 1) obtained from the usual dissociation expression:

$$\frac{(P-E)(C-E)}{(E)} = K, \qquad (1)$$

where P = total protein, C = total coenzyme, E = active enzyme, $\frac{P}{C}$ = constant, and K, a constant.

However, the addition of a boiled and filtered solution of the pure enzyme to dilute β-glucuronidase produces a strong activation (Table 1). This behavior is consistent with the view that spleen β-glucuronidase contains a thermostable coenzyme that dissociates on dilution. Furthermore, in the presence of 0.3% desoxyribonucleate the activity of pure β-glucuronidase is proportional to the protein concentration; i.e., E/P becomes independent of the protein concentration (black dots, Fig. 1). The distance on the ordinate between open circles and black dots indicates the activation by DNA for each protein concentration. This is very considerable at low protein concentrations.

TABLE 1

Protein concentration (mg/ml)	Activity / Protein = E^*/P	$\frac{E}{P}$ in the presence of boiled enzyme†	Activation by boiled enzyme
0.08	0.46	...	.....
.025	.23	0.41	1.8 fold
.02	.17	.44	2.6 "
.015	.11	.37	3.4 "
0.01	0.064	0.38	6 "

* The ratio E/P is given the value 1 when 0.165 mg phenolphthalein glucuronide is hydrolyzed in 1 min at 37° C by 1 mg protein in a digest of 1 ml.

† The concentration in the digest of the thermostable matter added with the boiled and filtered enzyme corresponds to 0.08 mg protein/ml before boiling.

When the activity of a dilute solution of pure glucuronidase (0.01 mg protein per ml) is determined in the presence of increasing amounts of desoxyribonucleic acid (DNA), and when the ratio of the enzyme activity to the protein concentration is plotted against the log of the DNA concentration, another S-shape curve is obtained. This experimental curve is in accordance with equation (1) when C becomes $(C+N)$, and N = concentration of DNA. This generalized equation also explains why E/P becomes independent of the protein concentration in the presence of a large excess of DNA (Curve 2, Fig. 1).

Accordingly, the addition of DNA in this system has the same effect as if more of the coenzyme were being added. However, we have no evidence that DNA is indeed the coenzyme. Yeast ribonucleic acid (RNA) gives a similar but somewhat smaller effect. For both DNA and RNA the activating effect is independent of their degree of polymerization.

[1] Aided in part by an institutional grant from the American Cancer Society, New York City, and the Cancer Institute, National Institutes of Health, U. S. Public Health Service, Washington, D. C.

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C^{14} Labeled 4(5)-Amino-5(4)-Imidazolecarboxamide in the Biosynthesis of Purines[1]

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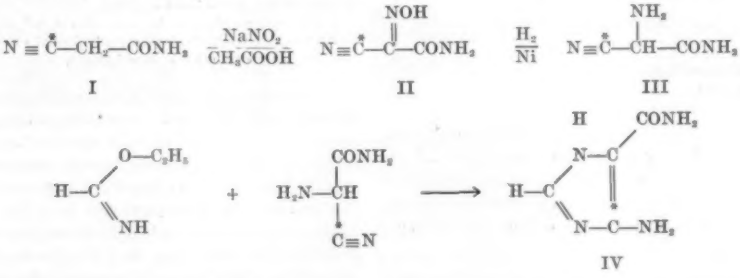
In a recent report from this laboratory (*1*), it has been shown that pigeon liver homogenates can convert 4(5)-amino-5(4)-imidazolecarboxamide (IV) to hypoxanthine, whereas the intact pigeon transforms the substance into uric acid. As part of a general program to study the biosynthesis of purines from labeled precursors, we have developed a convenient synthesis of 4(5)-amino-5(4)-imidazolecarboxamide containing C^{14} in the 4(5) position. Recently two new syntheses of this compound (nonlabeled) have been reported (*2, 3*).

Sodium cyanide (6 g) containing 0.6 mc of radioactivity as C^{14} and chloroacetic acid (12.7 g) were converted to cyanacetamide (I) by a modification of procedures described in *Organic Syntheses* (*4, 5*). The resulting labeled cyanacetamide was diluted with nonlabeled cyanacetamide (total, 16.75 g) and subsequently nitrosated with sodium nitrite in dilute acetic acid at 10°–15° to give labeled nitrosocyanacetamide (II) (17.4 g). The nitrosocyanacetamide was reduced with hydrogen and Raney nickel catalyst in methanol solution to the corresponding unstable aminocyanacetamide (III), which was used immediately in the next step without isolation. The addition of ethyl formimino ether in 20% excess to the cold methanol solution of aminocyanacetamide gave the desired 4(5)-amino-5(4)-imidazolecarboxamide (IV)



which was isolated and purified as the picrate (9.5 g pure); mp, 239°–240° C decomposition (cor.) when inserted in a bath preheated to 227°.

Analysis calculated for $C_{10}H_9O_8N_7$: C, 33.81; H, 2.56; N, 27.58. Found: C, 34.00; H, 2.51; N, 27.68.[2]

The picrate was conveniently converted to the hydrochloride by suspending it in dry acetone and rapidly saturating with dry HCl while maintaining the temperature constant; mp, 262.5°–263.5° decomposition (cor.) when inserted in a bath preheated to 245°.

Analysis calculated for $C_4H_7ON_4Cl$: C, 29.53; H, 4.34; N, 34.46; Cl, 21.83. Found: C, 29.78; H, 4.39; N. 34.30; Cl, 21.78.[2]

The picrate and hydrochloride respectively were found to be identical with samples of picrate and hydrochloride[2] prepared by the method of Windaus and Langenbeck (*6*).

A sample of the picrate was oxidized to carbon dioxide and assayed as $BaCO_3$ in the Geiger counter to give a value of 400 cpm/mg of carbon (calculated to infinite thickness) for the picrate. This corresponds to 1,000 cpm/mg of carbon for the free base.

To 5 rats of 155 g average weight was administered subcutaneously a total of 0.92 g of the 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride in 45 ml of solution half isotonic with respect to sodium chloride. Each rat received 1 ml of the solution three times a day for 3 days. The rats were kept in individual metabolism cages, and 24-hr collections of urine were pooled and kept in the cold room. The respiratory CO_2 contained negligible radioactivity.

The rats were killed on the fourth day (15 hr after the last injection), and the free nucleotides and the nucleic acids isolated by the procedure used in this laboratory. Adenine from the nucleotides and the 2 purines from the nucleic acids were isolated as the picrates, recrystallized, and converted to the hydrochlorides by suspending in acetone and saturating rapidly with dry HCl. Each fraction was separately purified by the chromatographic procedure of Cohn (*7*). The fractions were followed spectrophotometrically by comparing ratios of readings at 262 mμ and 248 mμ. A middle fraction containing a

[1] Aided by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council. One of us (C.S.M.) wishes to express his thanks to Sharp & Dohme, Inc., for financial aid. The C^{14} was received on allocation from the Atomic Energy Commission.

[2] We are indebted to K. B. Streeter, Edward A. McFadden, and Joyce L. Pyett, of the Organic Research Laboratory of Sharp & Dohme, Inc., for these analyses.

[2] We are indebted to Everett M. Schultz, of the Organic Research Laboratory of Sharp & Dohme, Inc., for a sample of the hydrochloride prepared by the method of Windaus and Langenbeck.

mixture of adenine and guanine was run through the column a second time. The fractions thus obtained were neutralized with concentrated NaOH and precipitated with $CuSO_4$ and $NaHSO_3$. The separated fractions were converted to picrates and purified to constant radioactivity by repeated recrystallization from 25% acetic acid. The counts were corrected to infinite thinness and are recorded in the table.

We found 39% of the administered 4(5)-amino-5(4)-imidazolecarboxamide in the pooled urines, using the colorimetric procedure of Bratton and Marshall (*8*). Of this amount, at least 13% was present in a nondiazotizable form, which was freed on mild HCl hydrolysis.

Isolation and separation of the allantoin and 4(5)-amino-5(4)-imidazolecarboxamide from the urine were effected by the allantoin procedure of Valentine, Gurin, and Wilson (*9*), involving $AgNO_3$ and NH_4OH precipitation (the imidazole compound precipitated first) followed by mercuric acetate precipitation of each of the fractions. There was obtained 0.69 g of the crude imidazole picrate which, on purification, showed no significant difference in radioactivity from a sample of the picrate of the administered material. The allantoin (0.274 g) was purified to constant radioactivity after treating with Dowex 50 to remove any basic material. The radioactivities are shown in Table 1.

TABLE 1

	Compound	Cpm/mg comp. inf. thinness
1	4(5)-amino-5(4)-imidazolecarboxamide picrate	1,160
2	4(5)-amino-5(4)-imidazolecarboxamide picrate from urine	1,110
3	Nucleic acid guanine picrate	103
4	Nucleic acid adenine picrate	134
5	"Nucleotide" adenine picrate	122
6	Allantoin	482

The carbon of the 4(5) position of 4(5)-amino-5(4)-imidazolecarboxamide appeared in the adenine of the nucleotides and the adenine and guanine of the nucleic acids, as well as in the allantoin of the urine. Some of the unchanged imidazole appeared in the urine with its radioactivity undiluted. This indicates there is no appreciable pool of this material in the body of the rat and suggests that the imidazole compound is not formed as a normal intermediate.

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An Improved Water Separator

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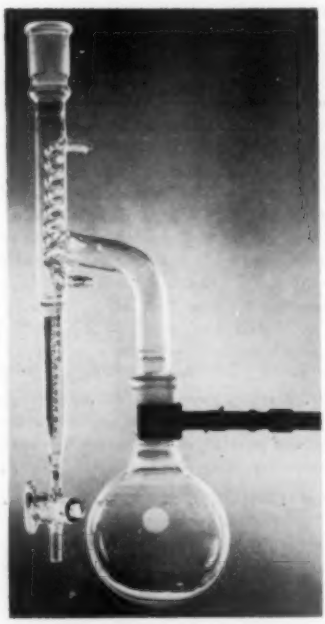
Many reactions in chemistry involve the removal of water from a reaction mixture through azeotropic distillation with a solvent lighter than water such as benzene or toluene. The water separates from the condensate as it cools. Most of the devices used for this purpose, such as the Dean-Stark receiver, return the condensate through hot vapors to a reservoir where the water separates. We have found that cooling the material in the reservoir greatly hastens the collection of water, and therefore have modified the condenser so that part of the cooling surface is in contact with the condensate, keeping it cold through the help of convection currents. The built-in spiral condenser (see figure) is more compact than the ordinary



type. The reservoir, which is made from a graduated centrifuge tube, has a stopcock that permits the use of the apparatus for automatic separation of large quantities of water when a large-scale condensation is done, since an inverted L of rubber or glass tubing attached at the stopcock can be arranged as an automatic water takeoff. A ground joint above the spiral gives a place for an additional condenser if a very rapid reflux is needed.

Comments and Communications

Scientific Ethics

In the article by Pigman and Carmichael in SCIENCE (1950, 111, 643–47), on page 645 is the suggestion that scientists in general should follow the principles of medical ethics and ban all advertising and publicity-seeking by scientists. This policy has certain drawbacks when applied to the commercial consulting scientists who deal directly with the public, usually with business enterprises.

The medical code was drawn up primarily because the medical profession deals with human illnesses, and there is need to prevent the exploitation of individuals in their time of trouble. The work of the consulting scientist who maintains his own office and laboratory is usually concerned with normal business problems and only rarely with the distress of an individual.

The consulting scientist is normally a specialist in the technology of a relatively narrow segment of industry. As a result his practice tends to be very widespread geographically. It is only by having a sizable number of such widely scattered clients that the modern consultant can maintain his investment in modern laboratory equipment. In order to build and hold such a clientele, practically all consultants have found it essential to maintain a sales program. This may include professional and trade paper advertising, direct mail contacts, and personal salesmanship.

It appears to be essential that the consulting scientist be permitted to advertise in an ethical manner. Any scientific code of ethics should make provision for this work. The exploitation of scientists by their employers' advertising of products is another problem which should receive attention. In this field there is much advertising of a questionable nature.

MAX C. MARKLEY

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What Dr. Markley may have in mind is perhaps illustrated in another letter, which was not submitted for publication, but part of which the editors would like to quote, without revealing the identity of the author:

There is one serious situation in which the industrial scientist on the staff of commercial organizations frequently finds himself. This is when the advertising man decides he wants to run a series of technical ads blowing up the technical know-how and control back of the product.

The normal course is for the advertising man to notify the chemist that he is coming over with a photographer and to be dressed in the proper white uniform and to have the laboratory shining. Then he wants the chemist to pose with an impressive but meaningless collection of glassware. The resulting pictures are then used in display advertising.

The chemist cannot refuse, because he would be guilty of failing to cooperate, and would then lose his position. At the present his only defense is to prevail upon the advertising man to use the most photogenic of the girl lab assistants in his place. If there were a scientific code of ethics defining what is proper in advertising then this situation could usually be controlled by the chemist.

The Use of Sodium Propionate in Isolating Actinomycetes from Soils

The problem of isolating pure cultures of actinomycetes from soil and other heterogeneous samples has never been satisfactorily solved, because the bacteria and molds soon overgrow the slower-growing actinomycete colonies. The use of special media favoring growth of actinomycetes helps but does not solve the problem; neither is the use of inhibiting dyes a success. Since, in our laboratory, research is being done on the isolation and identification of actinomycetes, it was decided that some method of inhibiting other organisms on soil plates should be studied.

The success of the baking industry in reducing mold contamination of bread and other bakery products by the use of sodium propionate, and the discovery by one of our graduate students that the growth of actinomycetes is not adversely affected by sodium propionate, suggested that the incorporation of this compound into actinomycete media might prevent overgrowth of actinomycete colonies by undesired organisms.

To test the efficacy of such a treatment, therefore, glucose asparagine agar, containing various concentrations of sodium propionate, was prepared. The concentrations tested ranged from 0.1% to 6.4%. Treated and control media were used to plate out a 1 : 10,000 soil dilution. These were incubated at 25° C and observed, and results were recorded after 48-, 72-, 96-, and 144-hr intervals.

It was found that concentrations of sodium propionate above 0.8% inhibited actinomycetes, as well as the undesired microorganisms. A concentration of about 0.4% sodium propionate seemed to be optimum for actinomycete isolation. Although both bacteria and molds grew at this concentration, the rate was sufficiently retarded to allow for the growth and subsequent isolation of the actinomycetes. Even after 144 hr incubation the usual spreading types of bacteria and filamentous fungi did not overgrow the actinomycete colonies.

Our experience so far indicates that the above procedure allows easier isolation of actinomycetes, especially the slower-growing forms. As a result of using such treated media in plating certain soils, we have obtained actinomycetes that were not found when standard media were used.

A more detailed report will be given in the near future.

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Book Reviews

The Biology of Human Starvation, 2 vols. Ancel Keys *et al.* Minneapolis: Univ. Minnesota Press; London: Geoffrey Cumberlege, Oxford Univ. Press, 1950. 1,385 pp. $24.00 the set.

This is largely a report concerning the influence of a six-month's semistarvation experiment on 32 healthy young men with an average age of 25 years. The subjects were volunteers from the camps or groups of conscientious objectors in World War II. The food restriction resulted in a loss of about 25% body weight at the end of the period. This study, which has become known as the University of Minnesota Experiment, might be said to be a revised and enlarged, or World War II edition, of the Carnegie Nutrition Laboratory study of the effect of undernutrition on young men which was stimulated by World War I.

A great deal of valuable data on the morphologic, biochemical, physiological, and psychological aspects of semistarvation and rehabilitation was secured in the Minnesota experiment with techniques developed since World War I, but the over-all results are in some respects disappointing. The semistarvation diet used undoubtedly produced conditions like those produced by semistarvation in Europe in World War II, but the value of the study might have been considerably increased by using two or more types of restricted diets. At least the salt intake could or should have been on different levels. Some of the subjects questioned the competence of the experimenters to plan adequate dietary rehabilitation. In the absence of knowledge based on previous experience, it would have seemed wiser to depend partly on instinct by allowing half of the subjects relatively free choice after a day or two of controlled food increase. On the basis of known facts, the statement that the fat content of a rehabilitation diet is unimportant (p. 1057) is not justified. Moreover, the claim that hunger disappears after a few days of total starvation (p. 829) is contrary to our findings.

No attempt was made in the Minnesota experiment to make a detailed study of hunger and appetite, but it was found that hunger definitely increases with semistarvation and in the early stages of rehabilitation. An important discovery was the finding that the amount of interstitial fluid remains relatively constant in spite of a loss of about 25% body weight and the development of edema in some of the subjects, but the question remains whether this would also be found with other types of restricted diets. The proportion of body fat was found to rise above the pre-semistarvation level during dietary rehabilitation, and the question raised here is whether complete dietary rehabilitation can be secured in any way without the development of a transient excessive fat deposit or a complication with poststarvation edema.

The authors seem to overemphasize the differences between the results of nutrition studies made on animals and on man. Animal experiments have, however, contributed greatly to our present knowledge of foods and nutrition, and it is highly probable that many of the unsolved problems of human starvation will be clarified by animal studies. The attitudes developed by some of the subjects in the Minnesota experiment also indicate that it might be extremely difficult to obtain a group of human guinea pigs for such studies in peacetime.

As stated in the authors' foreword, one of their objectives was to present a critical analysis of all the world's knowledge concerning the biology of human starvation. With a flow of important new literature coming out of Europe, this proved to be practically impossible. These two volumes will nevertheless long serve as the chief source of information concerning human semistarvation, a chronic state of millions of people in many lands.

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A Geography of Europe. Jean Gottmann. New York: Holt, 1950. 688 pp. $5.00.

Readers of SCIENCE who have been wanting to get a better grasp on the geographic, economic, and political realities of present-day Europe would do well to double-star this volume. Although written primarily as a textbook, it will undoubtedly satisfy the needs of other serious students who feel that America's destiny is somehow enmeshed with that of our European cousins.

The appearance of a new textbook in regional geography is always an event of importance to college and university teachers. Particularly so is the publication of this first book on the geography of Europe to make its appearance since World War II. It is a worthy successor to the half dozen or so prewar texts on Europe published in the United States, two of which, like this one, were written by European-trained geographers. Even though the author of a book on the geography of an entire continent must of necessity depend largely on secondary materials, the organization, point of view, and treatment of detail are all benefited by careful training in, and wide observation of, the area being described. The excellent bibliography is indicative of the author's familiarity with French, Italian, German, and Scandinavian source materials as well as the best of the literature in English. Bibliographies are always easy to criticize, but this reviewer cannot refrain from wondering why a two-volume work on "*Hochbulgarien*" is included when there is no mention of such standard German works as the *Handbuch der Geographischen Wissenschaft* or the European volumes in the *Enzyclopedie der Erdkunde* series.

After devoting 90 pages to the general features of Europe, in which he presents with sweeping strokes the physical and cultural-economic patterns of the continent as a whole, Gottmann analyzes the geographic "personality" of the individual countries. He groups these countries as belonging to four large regions: Western

Europe, Central Europe, Mediterranean Europe, and Eastern Europe, placing in the last of them only European Russia. Although in general Gottmann's subdivisions of the continent correspond quite closely to those of the masterful *Géographie Universelle* series, to which he recognizes his indebtedness, this reviewer sees little point in placing Rumania and Bulgaria in two separate regions, or in detaching the treatment of Finland completely from that of Scandinavia, with which it has so many obvious affinities. In view of the present availability of textbooks on the geography of the entire USSR, it appears rather unnecessary to perpetuate the fiction of a boundary at the Urals by including a treatment of European Russia.

The chapters on individual countries are well written, with a fine balance between physical considerations (landforms, climate, soil, etc.) and human factors such as population density, economic development, and political experience. France and Scandinavia are particularly well handled. There are occasional lapses into loose writing. Without defining terms it is confusing to write that "in Stockholm winter lasts 121 days, spring 57, summer 124, and autumn 63" (p. 122). It is incorrect to describe Luxembourg as being located "in the heart of the Ardennes Plateau" when only about one-third of the territory and probably not one-tenth of the population of the Grand Duchy live in the area of Paleozoic bedrock (p. 246). A few misprints were noted which can easily be corrected in later printings. The photographs are excellent, and good use has been made of numerous maps originally published in the *Geographical Review*.

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Gray's Manual of Botany: A Handbook of the Flowering Plants and Ferns of the Central and Northeastern United States and Adjacent Canada. 8th ed. Revised by Merritt Lyndon Fernald. New York: American Book, 1950. 1,632 pp. $9.50.

This new centennial edition follows the first after an interval of 102 years, and the preceding seventh edition after 42 years. The last edition actually prepared by Asa Gray was the fifth (1867). Since then the successive copyrights have been held by the president and fellows of Harvard College, and the revisions have been primarily the work of the staff of the Gray Herbarium. Needless to say, the changes have been so extensive that each of the last three editions has been essentially a new work, although retaining some features of its predecessor.

M. L. Fernald, who largely revised this edition, had already put the indelible mark of his personality into the seventh, which was the joint effort of himself and his colleague B. L. Robinson. It was Fernald who had the zeal and physical aptitude for field work which resulted in important botanical explorations of Quebec and Newfoundland and the extension of the range covered by the seventh edition of the *Manual* northward to cover the area below the Straits of Belle Isle and from Anticosti Island westward along the 49th parallel in Quebec to the northwestern corner of Minnesota. Southward from that point the "Gray's Manual region," unchanged since the former edition, follows the western boundaries of Minnesota and Iowa and then the 96th meridian. The southern boundary, likewise unchanged, follows the southern boundaries of Virginia, Kentucky, Missouri, and eastern Kansas. Nebraska and Kansas are thus the only states whose floras are but partially covered.

Although Fernald devoted almost all his energy to botanical exploration of the "Manual" area and revision of the *Manual* in the 42 years after the seventh edition, he had the help of some 400 collaborators, whose problems and questions all had to be to a high degree satisfied by his conclusions. Probably no botanical systematist has ever before gone so far in satisfying so many active finders of deficiencies and faults in a standard flora! Fernald has been an inspiring leader, and during four decades botanists of the "Manual" region have looked forward to "the new Gray" as though it would be oracular. There was a constant outpouring all this time of from one to several articles a month from Fernald's pen, mostly in *Rhodora*, the journal of the New England Botanical Club, of which he became the editor after Robinson's death, so that it came to be one of the most highly personalized of scientific journals, in an era in which most editors have deemed it scandalous to reveal any personality at all. *Rhodora* indicated, as the years went by, more and more of what would eventually be summarized in the *Manual*.

Fernald sought places for his own field work where there were the best chances of significant discoveries, and where workers were few. At first his efforts were devoted largely to Gaspé and Newfoundland. Having stimulated local zeal in further development of floristic knowledge of the northern border, he turned to the coastal plain of Virginia, and made a multitude of interesting discoveries. Moreover, he was ever ready for a trip to some neglected place within a short distance of Cambridge and even there he often found something that others had overlooked. When his eyes became impaired there was general lamentation, and fears were expressed that perhaps the *Manual* would become a casualty, but with the loyal aid of Bernice G. Schubert he valiantly pushed it along to a conclusion.

The *Manual* carries on the old tradition of recognizing many systematic entities as varieties and forms. Fernald did not follow the recent tendency either to consider everything as a species if it could be distinguished at all, or to require that every distinguished subspecific entity have a distinctive geographic range. Although he was ever a keen student of geographic distribution, he showed little regard for "life-zones," which in practice cannot be usefully defined or delimited.

Fernald likewise had no use for spurious common names, made by translating scientific names into English, and had the courage of his convictions in refusing to use them. The furtherance of "standardized plant names" gets no encouragement from the new *Manual*. To be recognized, common names must somewhere belong to common language. Fernald was, however, a critical student of real vernacular names, as is evidenced in the

Manual by his careful introduction of Canadian-French popular names for species that range into the French-speaking part of Canada. The utterly absurd run-together names which certain pedants seek to force upon us (Fernald gives "treeofheaven" as an example) have no acceptance. Those who like such pedantry as "standardized common names" won't like the new *Manual* any better than the old.

Another adherence to good tradition that will irk the strictly up to date among us is the retention of capital letters in spelling the second word of certain binomials, as, for instance, for substantives in apposition. All in all, Fernald has set himself against thoughtless fads and innovations, which have been largely adopted in imitation of the zoologists, and has held fast that which is good. In matters of Latinity he has had the advice of an incomparable classicist and able botanist, A. S. Pease, of Harvard.

It is interesting to see what has been done with notoriously difficult groups. Fernald has replaced the treatment of grasses, contributed to the former edition by A. S. Hitchcock, by one of his own, more concordant with past *Manual* usage in the reduction to varietal status of many entities which Hitchcock would have maintained as species or neglected entirely. In spite of the application of this policy in the reduction of species to varieties in the difficult genus *Panicum*, new specific proposals and extensions of range increase the number of species from 73 in the seventh edition to 76 in the eighth, and many additional varieties are accounted for. This is just one example of the amazing expansion of the present work from the 4,885 named entities of its predecessor to 8,340.

It was, of course, C. S. Sargent at Harvard who initiated the vast increase in species of Crataegus. The Gray Herbarium, however, remained aloof while the stampede of naming went on at the Arnold Arboretum. When the genus had to be covered for the seventh edition, the job was done by an outsider, the late W. W. Eggleston, of the Bureau of Plant Industry. The disregard of Crataegus at the Gray Herbarium remained the same when the present edition was prepared, and, in Fernald's words, we are presented with "a treatment of the intricate genus Crataegus prepared by the only student who professes to understand it, Ernest Jesse Palmer," of the Arnold Arboretum. Crataegus has been increased from the 65 species recognized by Eggleston to 103 that are considered to be properly attested, together with a large group of others which are only tentatively admitted as "trees or shrubs of very limited occurrence or of suspected hybrid origin." The latter are interpolated where they appear to belong, systematically, but without numbers, and are not taken into account in the keys. Students of local floras wishing to contribute to a clarification of the systematics of Crataegus will do well to compare Palmer's *Manual* treatment with his "Synopsis" of North American Crataegi in Vol. VI of the *Journal of the Arnold Arboretum*. In view of the uncertainties regarding the true geographic ranges of the Crataegi, knowledge of the type localities, given in the "Synopsis" but not in the *Manual*, is very useful indeed.

Turning to Rubus, a prime puzzle to European systematists for generations, but only seriously engaging the attention of American botanists during the past 50 years, we find a vastly expanded revision, in which Fernald considers that he has been too liberal in acceptance of numerous propositions of L. H. Bailey. Here the admitted species are 119 as contrasted with 38, and still others are refused recognition which have as good claim to admission as some that have been accepted. It will be interesting to see how workable the new treatment will prove to be in years to come.

Many other "critical" genera have been interestingly dealt with, but lack of space forbids mention of them all. No one, however, will expect the present reviewer to fail to refer to the treatment of *Oenothera biennis* and its allies. Here Fernald frankly gave up the task of attempting to bring order out of chaos, and the trouble was not that too little was known of the group, but, rather, too much. The findings of geneticists have been largely disregarded by the systematists, and vice versa, although R. R. Gates and others have tried to make some application of genetic findings to classification. Efforts that have been made, however, have not been at all effective. To quote Fernald, we deal with "A hopelessly confused and freely hybridizing group, early introduced into Europe, and there cultivated, and, like other plants of the garden, intermixed; then spreading to waste or open ground. The types of several species, described from European material, not wholly clarified; and further confusion added by the publication of many scores of 'mutants' or 'elementary species' as true 'species.' . . . These, for the most part, not specially considered here."

Of course Oenothera is *not* apomictic, and is comparable in this respect to Taraxacum, as Fernald implied, but quite the contrary. The very large-flowered types of the subgenus Onaga freely hybridize. Fernald unites those of the East (excepting *Oe. argillicola*) as *Oe. biennis* var. *grandiflora*, disregarding the laborious experiments of B. M. Davis in which there was failure in the effort to simulate *Oe. Lamarckiana* closely by crosses into which *Oe. grandiflora* entered as a parent. Utter submergence of *Oe. Lamarckiana* as a synonym of what is conceived of as a variety is indeed a drastic and summary way to dispose of the experimental background of De Vries' mutation theory! It would have been more realistic and more in accord with the attitude adopted in dealing with Rubus, Antennaria, Crataegus, Panicum, Viola, and other difficult groups if a number of representative types which experimentation has shown to exist in nature had been recognized. The treatment in the new *Manual* is little changed from that of its predecessor. Perhaps Cleland's general review of Oenothera genetics, which is now being looked forward to, will present cytogenetic findings clearly enough so that systematists can profit by them. If so, Fernald's withdrawal from the problem may have been the wisest course.

It is not to be expected of such a vast labor of revision as the total *Manual* that in each detail it will satisfy everyone. On the whole, however, it is a highly satisfactory and noble achievement, the culmination of a lifetime devoted to the reinterpretation of our flora, as largely on the basis of zealous personal field work as half

a century would permit. We are entering a period in which the experimental investigations of geneticists, cytologists, and biochemists are coming to have more and more bearing upon classification, and it will soon be impossible for one botanist to have critical firsthand knowledge of so large a flora as Fernald had. His book will be a lasting landmark in the botanical history of our region, and it is a source of deep satisfaction to his devoted botanical following that he lived to see it in print.

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Biological Actions of Sex Hormones. Rev. 2nd ed. Harold Burrows. New York: Cambridge Univ. Press, 1949. 615 pp. $8.50.

The rapid and continuous progress made by investigators in endocrinology has caused many books in this field to become out-of-date shortly after publication. Consequently, prompt revision is not only an ideal goal but a definite necessity. The second edition of *Biological Actions of Sex Hormones* has been revised and enlarged by approximately 100 pages since it first appeared in 1945. Thus, within a matter of four years the advances in our knowledge of sex hormones has led Burrows to expand his book by approximately 20 per cent.

As with the first edition, this volume includes an excellent bibliography, which has been enlarged to 3,000 references. This alone would make the book a "must" for students of the sex hormones. Dr. Burrows has retained the general form and organization of the first volume. The book is divided into six parts, each part containing one to ten chapters. In Part I the author discusses the gonadotrophins, their action, factors influencing their release, etc. In Part II a general consideration of the gonadal hormones is given as an introduction to the main portion of the text. The remaining sections are devoted to consideration of the sex steroids and their actions. The book is well written, reads smoothly, and the text is generously interspersed with tables, which add greatly to its value. There is, however, little attempt at interpretation and correlation of the facts, and a critical evaluation of the reported data would greatly enhance the book's value.

Reference to relaxin is included for the first time, but it is to be regretted that its role as a possible hormone of pregnancy (at least in some species, such as the guinea pig and mouse) was not stressed. Nomenclature is always a sensitive item with scientists, and the coining of new words is to be lamented when others are in universal usage. The use of FRH for FSH merely clutters up the field. A number of minor errors of commission and omission are to be found, but they are amazingly few. For example, the author fails to point out that adrenal hypertrophy does not always occur after estrogen treatment but seems to be dependent on the age of the animal. More space could be given to vitamin-hormone relationship, especially the role of folic acid.

A major criticism of this volume is the failure of the author to summarize the available data into working hypotheses for such phenomena as menstruation, estrous cycle, ovulation, pregnancy, lactation, etc. Thus, the subject of the interaction of all the hormones in the physiology of reproduction is not treated as such. In spite of this major criticism the second edition of *Biological Actions of Sex Hormones* is well on its way to becoming a standard reference book for students and investigators of the physiology of the sex hormones. It contains a wealth of well-annotated experimental data and it should be of great value to all students beginning their investigations on the sex steroids. It is to be hoped that the author will see fit to revise his book often. Failure to revise Allen's *Sex and Internal Secretions* since 1939 has made Burrows' book one of the major up-to-date works in this field.

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Scientific Book Register

Researches on Fungi: The Sexual Process in the Uredinales, Vol. VII. A. H. Reginald Buller. Toronto, Canada: Univ. Toronto Press; London, Engl.: Geoffrey Cumberlege, Oxford Univ. Press, 1950. 458 pp. $13.50.

Renal Function. Transactions of the First Conference, October 20–21, 1949. Stanley E. Bradley, Ed. New York: Josiah Macy, Jr. Foundation, 1950. 172 pp. $2.50.

Wave Theory of Aberrations. H. H. Hopkins. New York: Oxford Univ. Press, 1950. 169 pp. $3.00.

Emotions and Clinical Medicine. Stanley Cobb. New York: Norton, 1950. 243 pp. $3.00.

Photons and Electrons. K. H. Spring. London: Methuen; New York: Wiley, 1950. 108 pp. $1.75.

Experiments in Physical Chemistry. Otto F. Steinbach and Cecil V. King. New York: American Book, 1950. 250 pp. $3.50.

Encyclopédie Entomologique: La Biologie des Diptères, Vol. XXVI. E. Séguy. Paris, France: Paul Lechevalier, 1950. 609 pp. 4,000 fr.

Unit Operations. George Granger Brown, *et al.* New York: Wiley; London: Chapman & Hall, 1950. 611 pp. $7.50.

Scientific, Medical, and Technical Books Published in the United States: Supplement of Books Published 1945–1948. Prepared under the direction of the National Research Council's Committee on Bibliography of American Scientific and Technical Books. R. R. Hawkins, Ed. New York: R. R. Bowker, 1950. 514 pp. $10.00.

Some Applications of Statistics to Archaeology. Service des Antiquités de l'Egypte. Oliver H. Myers. Cairo, Egypt: Government Press, 1950. 37 pp. and 19 figs. Under 25/–.

Apples and Apple Products. R. M. Smock and A. M. Neubert. New York: Interscience, 1950. 486 pp. $7.80.

News and Notes

Conference on Ionospheric Physics

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A conference on ionospheric physics was held at The Pennsylvania State College July 24–27, with an attendance of 236. Twenty technical papers were presented by leading workers in the field from all parts of the world. The conference was sponsored by the School of Engineering of The Pennsylvania State College and the Geophysical Research Directorate of the Air Force Cambridge Research Laboratories. The enthusiasm with which the program was received was very gratifying to the sponsors.

A general survey of the present status of our knowledge of the "Physics of the Upper Atmosphere" was given by S. K. Mitra, of Calcutta, India, and this paper served as a starting point from which subsequent papers could logically proceed.

An extremely interesting, though controversial, subject was discussed by Leiv Harang, of Norway, in his paper, "The Constitution of the Upper Atmosphere as Determined from Auroral Studies." Aurorae have always been of interest to scientists concerned with the study of the upper atmosphere and the ionosphere, and the discussion subsequent to Dr. Harang's paper indicated that existing theories of the origin and effects of the aurorae are far from being universally accepted at present.

The use of rockets as instruments for the investigation of the characteristics of the upper atmosphere has been adopted since the close of World War II, and papers by T. R. Burnight, of the Naval Research Laboratory, and E. W. Beth, of the Air Force Cambridge Research Laboratories, disclosed the progress that has been made in the rocket study programs since their conception. Scientists in the field of upper atmospheric physics have eagerly awaited the results of the rocket measurements—the first ever made by actually having the measuring apparatus at the height of the various ionospheric layers. It was gratifying to note the high degree of agreement obtained between the rocket measurements and previous radio measurements.

Sidney Chapman, of Oxford, England, presented a paper on "Corpuscular Influences upon the Upper Atmosphere." His presence at the conference was enjoyed by all, and more than one reference was made to his invaluable work on the effects of solar radiation on the upper atmosphere.

Some very worth-while work has been conducted in Australia during recent years, and three speakers who took part in the conference have been responsible for most of it. D. F. Martyn, of Canberra, contributed two papers on the dynamics of the upper atmosphere and served as discussion leader for a symposium on the same topic. L. G. H. Huxley, of Adelaide, also presented two papers, one on "The Motions of Very Slow Electrons in Air" and the other on "Ionospheric Cross Modulation." R. v. d. R. Woolley, of Canberra, gave a paper on the "Connection between the Solar Corona and Chromosphere and the Ionosphere."

A considerable portion of the conference was devoted to the important but vexing problems of ionization and recombination processes in the upper atmosphere. Several of the foremost authorities expressed their views on the matter. D. R. Bates, of London, England, J. Sayers, of Birmingham, England, Peter M. Millman, of Ottawa, Canada, and M. H. Johnson, of the Naval Research Laboratory, each presented papers concerning the formation and disappearance of ions and electrons and diffusion in the ionosphere.

Somewhat akin to this subject was the paper "Ionosphere Winds" presented by K. Weekes, of Cambridge, England. Considerable discussion on similarities and differences in methods of measuring winds in the ionosphere and the results obtained from these measurements ensued.

A problem that often comes to the fore in the study of the ionosphere by means of radio waves is that of scattering. Some new ideas were presented by H. G. Booker, of Cornell University, in his paper "Scattering of Radio Waves with an Application to Radio Astronomy." W. Dieminger, of Lindau, Germany, discussed the "Origin of Ionospheric Scattering."

The E layer of the ionosphere has been said to be the most well-behaved of all ionospheric layers, and the one about which we know most; yet, when it is considered in any detail, the existence of many complexities and abnormalities becomes immediately apparent. For this reason, several papers concerned with this topic were invited. The foremost was a paper entitled "Studies of Ionospheric Region E," by W. Pfister, of the Air Force Cambridge Research Laboratories. The paper by M. Nicolet, of Uccle, Belgium, on the "Effects of the Scale Height Gradient on the Variation of Ionization and Absorption" was also appropriate, not only in connection with the E region but also with the problem of ionization in general.

A change from the technical sessions was provided by the after-dinner talk of Lloyd V. Berkner, of the Carnegie Institution of Washington, on "Signposts to the Future of Ionospheric Research."

The high lights of the entire conference were the three symposia held for the purpose of exchanging ideas on recent developments and controversial points. All the topics presented in the papers, and more, were discussed to some extent in these meetings, and it was generally agreed that the symposia, because they allowed free intermingling of ideas and discussions in a somewhat informal atmosphere, were a real success.

Scientists in the News

Recent visitors at the National Bureau of Standards from abroad were: **S. Amari**, Radio Regulatory Commission, Tokyo; **F. L. Brady**, Marley Tile Co., Ltd., Sevenoaks, Kent, England; **Shinzo Okada**, Kyoto University, Japan; and a Swedish plastics group sponsored by the ECA.

F. Warren Bickel, technical director of the Diamond Wire and Cable Company, has been named project leader in a new rubber research laboratory at Armour Research Foundation of Illinois Institute of Technology. In his new position, Bickel will direct compounding operations and evaluation work with the various rubbers and elastomers. He will work with **Alfred G. Susie**, supervisor of rubber and plastics research.

Sigfried Bjerninger, of the Mechanical Engineering Institute of Agricultural Engineering in Sweden, recently visited the Davis campus of the University of California. He is in the U. S. under ECA sponsorship to make a study of wagons, trailers, tractors, blowers, and elevators, pumps for irrigation and for home water supply, automatic water supply systems, hydraulic lifts, and other tools for work simplification. **Costa Tarlatzis**, Department of Diagnosis, Veterinary Bacteriology Institute, Greek Ministry of Agriculture, also visited the Davis campus. Dr. Tarlatzis is studying American methods in veterinary bacteriology and control of animal diseases. **T. O. Bacher**, director of the National Greenhouse Experimental Service of Denmark, has been at Davis to study the work being done there on vegetables, small fruits, and flowers.

Walter M. Boothby, professor emeritus of the Mayo Foundation and a leader in medical research, has joined the staff of the School of Aviation Medicine, Randolph AFB, as research advisor. Dr. Boothby has been advisor on research in aviation medicine and physiology to the Swedish Aviation Medicine Council at the University of Lund for the past two years.

Harry Woodburn Chase, chancellor of the New York University since 1933, will retire on July 1, 1951, at the completion of his 32nd year as a major university administrator —one of the longest records of any figure in the history of American education. He also served as president of the University of North Carolina from 1919 to 1930 and as president of the University of Illinois from 1930 to 1933.

David Davis has joined the staff of the Connecticut Agricultural Experiment Station's Plant Pathology Department, replacing **Richard A. Chapman**, who resigned to become associate plant pathologist at the Kentucky Agricultural Experiment Station, Lexington. Dr. Davis will join the group of station researchers working on the chemotherapy project and will concern himself especially with its application as a control for the Dutch elm disease. One of Dr. Chapman's first projects at the Kentucky station will be an attempt to find a control measure for a root rot attacking the brace roots of corn.

The U. S. Geological Survey reports the arrival of **H. J. Evans**, mining engineer, of Zinc Corporation, Ltd., Melbourne. He will visit in Texas, California, New Mexico, and western Canada until January 1, studying recent developments in the potash, sulfur, oil, and gas industries.

John S. Lea, formerly with the Engineer Research and Development Laboratories, Corps of Engineers, Fort Belvoir, Va., has been appointed editor of the U. S. National Museum, Smithsonian Institution, succeeding **Paul H. Oehser**, who was recently made chief of the Smithsonian's Editorial Division.

Reginald D. Manwell, professor of zoology, Syracuse University, and **J. Fisher Stanfield**, head of the Botany Department, Miami University, were recently elected members of the board of trustees of The Rocky Mountain Biological Laboratory. **A. O. Weese**, professor of zoology, University of Oklahoma, was reelected president of the board for the 14th consecutive year, and **John C. Johnson** was continued as director, having held that position since the laboratory was organized in 1928.

Albert E. Marshall, past president, American Institute of Chemical Engineers, has resigned as vice president of Heyden Chemical Corporation, to open an office in the Industrial Trust Building, Providence, R. I., and resumed practice as a consulting chemical engineer.

Paul F. Russell, malaria adviser of the International Health Division of the Rockefeller Foundation and chairman of the World Health Organization Expert Committee on Malaria, is attending the WHO African Malaria Conference and a meeting of the committee, both in Kampala, Uganda, November 27–December 11. Following these meetings, at the request of WHO, he will also visit other areas of British East Africa, the Belgian Congo, French Equatorial Africa, and British West Africa. Dr. Russell will then establish his headquarters at the Superior Institute of Public Health, Viale Regina Margherita 299, Rome, Italy, as the International Health Division representative in Italy.

Charles H. Schauer has been appointed director of grants of Research Corporation, New York, to succeed **Robert R. Williams**. Mr. Schauer was formerly with the Philadelphia Electric Company, the Office of Scientific Research and Development, and the NDRC. For his work in the latter two positions he was awarded the Presidential Certificate of Merit. Dr. Williams, pioneer in the synthesis of vitamin B_1, the anti-beriberi vitamin, is now on a world tour seeking to better the nutritional status of rice-eating populations. On his return from the Orient next spring he will continue actively in the affairs of Research Corporation as a member of the Advisory Committee on Grants and as chairman of the corporation-administered Williams-Waterman Fund, of which he is co-founder.

John W. Taylor, president of the University of Louisville, has been appointed deputy director-general of Unesco. Dr. Taylor will take up his post in Paris on January 1.

Grants

Grants-in-aid of research in cardiovascular disease and related fields are being offered by the **Louisiana Heart Association.** Application blanks may be obtained from Dr. Russell Holman, chairman, Research and Fellowship Committee, Louisiana Heart Association, Inc., 1430 Tulane Ave., New Orleans. The deadline is *January 2*, and it is expected that the funds will be available July 1. Preference will be given to requests from Louisiana.

The U. S. Public Health Service has awarded $735,854 in teaching grants to U. S. medical schools for wider instruction of students in the best and latest methods for coping with heart ailments. The sum awarded in this program by the **Heart Institute** now amounts to $1,463,814. Psychiatrists, psychologists, nurses, social workers, and medical students in training will be aided by grants totaling $1,915,708 from the **National Institute of Mental Health** (USPHS). In addition, $1,179,003 will be made available to graduate students in the same fields. Twenty-one new mental health research grants, plus renewal of 44 earlier grants, were approved early in November.

Ten industrial grants-in-aid have been received by MIT for use in the extensive development program for education and research in broad areas associated with the technical interests of participating companies. Typical grant-in-aid agreements provide for an exchange of current concepts and techniques in fields of research of common interest. The agreements are an important part of a continuing program designed to stimulate professional associations of mutual benefit to industry and MIT in the advancement of technology. The two largest grants are $100,000 each for a five-year period. These came from the Anaconda Copper Mining Company and its subsidiaries, Anaconda Wire and Cable Company and American Brass Company, and from the Atlantic Refining Company. Other unrestricted grants were from Godfrey L. Cabot, Inc.—$50,000 over two years; Liquid Carbonic Corporation—$50,000 over five years; and North American Aviation—$50,000 over five years. Grants for work in specific fields are: Continental Oil Company—$25,000 for chemical engineering, geology, nuclear science and engineering, and electronics; National Dairy Products Corporation—$30,000 for biology and food technology; Saco-Lowell Shops—$20,000 over a two-year period for metal processing; Sperry Gyroscope Company—$15,000 for two fellowships in electronics and one in aeronautical engineering; and Sylvania Electric Products—$50,000 over five years, for electronics, nuclear science, and engineering.

The Office of Naval Research has made a grant of $5,179 to Joanne Starr Malkus, assistant professor of physics at Illinois Institute of Technology, for meteorological investigations. Primary purpose of the project will be a study of the life histories, structure, and behavior of cumulus clouds, to gain information on the early stages of the formation of such clouds and to make practical analyses of thunderstorms and violent gusts and drafts encountered by aircraft in later stages of cloud development.

A grant for olfactory research has been made to Northwestern University's Medical School by Airkem, Inc. Jules H. Masserman, associate professor of nervous and mental diseases, will conduct the work, which will deal with the olfactory components of neurosis and the influence and effect of smell upon individual reactions and impressions.

Fellowships

Information regarding the **Frank B. Jewett Fellowships** in the physical sciences, announced in SCIENCE, November 17 (page 608), should be obtained from M. B. Long, Secretary of the Jewett Fellowship Committee, Bell Telephone Laboratories, 463 West St., New York 14, and not from J. C. Boyce.

The **Atomic Energy Commission** has announced that 40 graduate students are now receiving training under the AEC special fellowship program in radiological physics. Twenty are at the University of Rochester, and the others are at Vanderbilt University. Fellows asigned to Rochester will complete their course with about two months of field training at an AEC installation. Those at Vanderbilt will move to the Oak Ridge National Laboratory after nine months at the university.

Colleges and Universities

A virus research laboratory which will concentrate upon childhood infections has been established at the **University of Louisville School of Medicine.** Its completion was effected by a grant from the recently organized Kentucky Child Health Foundation. Investigations will be supervised by Alex J. Steigman and James G. Shaffer, of the school's Child Health and Bacteriology Departments.

A new postgraduate course in the "airbrasive" dental technique for drilling teeth will be offered by the **University of Michigan School of Dentistry,** beginning January 8. The new method substitutes for the dentist's drill a stream of aluminum oxide powder shooting out of a small nozzle under pressure from carbon-dioxide gas, at a speed of 1,000 feet/second. This method is only supplemental to the present drilling methods, as there is some dental work where it cannot be used. The School of Dentistry plans to give seventeen consecutive one-week courses limited to 16 members each. The courses will be open to any licensed dentist. A fee of $100 will be charged Michigan residents; nonresidents will be charged $150. Further information may be obtained from Dr. William R. Mann, School of Dentistry, University of Michigan, Ann Arbor.

The fifth annual John O. McReynolds Lecture in Ophthalmology was given by Arlington C. Krause, Department of Ophthalmology, University of Chicago, at the **University of Texas Medical Branch,** Galveston, November 6. This lectureship was established in honor of the late John O. McReynolds, a well-known Dallas ophthalmologist, by his daughter, Mrs. Frank W. Wozencraft. Dr. Krause discussed some of

his recent experimental studies on eye injury. Arthur Purdy Stout, professor of surgical pathology, Columbia University College of Physicians and Surgeons, gave a series of lectures at the Medical Branch during the last week in October on "Tumors of the Soft Tissues" and on "Changing Concepts of Malignancy." Dr. Stout also gave a series of special lectures on cancer at Brooke General Hospital, San Antonio. Peter Gaillard, professor of cytology at the State University of Leyden, Holland, gave a special series of seminar discussions during the first two weeks of November on the cultivation of whole organs. Dr. Gaillard is doing special work in the Tissue Culture Laboratory of the Medical Branch in association with Charles M. Pomerat, director of the laboratory.

The University of Michigan's School of Public Health is offering an in-service training course in radiological health, February 5–8. The course is designed especially for health officers, engineers and sanitarians, industrial hygiene personnel, and nurses. The enrollment fee is $5.00. Applications should be sent to H. E. Miller, School of Public Health, University of Michigan.

In the Laboratories

A new and exceptionally strong steel has been developed by the research laboratory of **Carnegie-Illinois Steel Corporation.** With good resistance to corrosion, it remains tough even at temperatures below zero, where many structural materials tend to show some loss of toughness. Bearing the grade designation "T1," this new Carilloy steel composition is almost twice as strong as the high-strength, low-alloy steels and will effect savings in mobile equipment, since it can be made in quantity in standard open-hearth furnaces. It may be welded easily with welding electrodes now available, yet it possesses almost three times the strength of ordinary welding-grade structural steels.

The Naval Research Laboratory is seeking scientists and technicians to fill 46 positions, ranging in classification from GS-7 to GS-12. Civil Service status is not required. The list includes 13 physicists, 22 electronic scientists, 4 electrical engineers, and a few chemists or chemical engineers and technicians. Information regarding these positions may be obtained from Personnel Division, Code 1817, Naval Research Laboratory, Washington 25, D. C.

Meetings

A symposium on **"Recent Advances in Microbiology,"** sponsored by the Department of Bacteriology and the Graduate School of Indiana University, will be held December 7, in the Alumni Hall of the university. Speakers on the program will be P. W. Wilson, University of Wisconsin, J. M. Sherman, Cornell University, H. O. Halvorson, University of Illinois, W. J. Nungester, University of Michigan, Jorgen M. Birkeland, Ohio State University, William Burrows, University of Chicago, and Morris Scherago, University of Kentucky.

Speaking before the 18th annual meeting of the **Engineers' Council for Professional Development** held in Cleveland, A. R. Hellwarth, Detroit Edison Co., said that industry invests at least $10,000 and two years of irreplaceable time before a newly recruited engineer is able to accept the full responsibility of a job in a modern industrial organization. "For this reason," said Mr. Hellwarth, "men and jobs must be well-matched if the investment is not to be a loss to industry." To aid both students and industry, a program of facts and suggestions has been incorporated in a 200-page compilation gathered from a 15-year study of the training programs of 54 companies and a review of the published information about programs of about 80 other firms by the ECPD committee on professional training, under the chairmanship of A. C. Monteith, vice president in charge of engineering and research, Westinghouse Electric Corporation, Pittsburgh. Raymond Walters, president of the University of Cincinnati and chairman of the liaison Committee on the Relationships of Higher Education to the Federal government, in his talk before the group said that serious financial problems in the colleges and a severe shortage of trained personnel in industry, especially scientists and technologists, can result from the drafting of large numbers of college-age youths.

The Western Forestry and Conservation Association will hold its annual conference at the Sir Francis Drake Hotel in San Francisco, December 6–8. Sessions of the conference will consider problems of growing, protecting, and harvesting the forest crop.

The 6th **Southwest Regional Meeting of the American Chemical Society** will be held in San Antonio, December 7–9, at the Gunter Hotel. Among the features of the program will be symposia on carbonium ions, chemical education, and radiochemistry.

A **Middle East Tropical Disease Symposium** was held November 18–19 at the American University of Beirut, under the auspices of the Division of Medicine of the university and sponsored by the United Nations Relief and Works Agency with the help of WHO, the U. S. Naval Medical Research Unit III, Cairo, and the French Faculty of Medicine, Beirut.

The American Chemical Society will hold a symposium on radioisotopes at the Hotel Statler in New York, January 19, 1951. Scheduled papers are: "Carbon 14 and New Frontiers of Organic Chemistry"—Charles E. Crompton, Atomic Energy Commission; "Nuclear-Inorganic Chemistry"—Charles D. Coryell, Massachusetts Institute of Technology; "Some Applications of Radioactive Isotopes in Biochemistry"—David Rittenberg, College of Physicians and Surgeons, Columbia University; "The Commercial Potential of Nuclear Radiation Sources"—F. C. Henriques, Jr., Tracerlab Inc.; "The Use of Radioisotopes in the Petroleum Industry"—Paul H. Emmett, Mellon Institute of Industrial Research, University of Pittsburgh; and "Principles of Radioisotopes Technology"—Charles Rosenblum, Merck & Co., Inc.

Vivid and authentic, based on first-hand information—

Geography of Russia

By N. T. Mirov, *University of California, Berkeley.* The author writes explicitly and specifically from first-hand information on location, tectonic structure, topography, glaciation, climate, rivers, and mountains. His discussion of the peoples of Russia includes subtle points on language and religion often missed by the superficial observer. The meat of the book is its detailed description of 20 important regions of the Soviet Union that includes examinations of the flora and fauna peculiar to each area. *January 1951. Approx. 334 pages. Illus. Prob. $5.00.*

Smoothes the transition to quantum and relativistic mechanics—

Classical Mechanics

By H. C. Corben, *Carnegie Institute of Technology,* and Philip Stehle, *University of Pittsburgh.* Has two chief objectives: to present classical mechanics in a way that exposes the underlying assumptions and indicates the boundaries beyond which its uncritical extension is dangerous; and to help the reader sense the continuity in physical thought as he advances from classical mechanics to quantum and relativistic mechanics. *November 1950. 388 pages. $6.50.*

From the standpoint of modern organic chemistry—

Heterocyclic Compounds, Volume II

Polycyclic Five- and Six-Membered Compounds Containing One O or S Atom.

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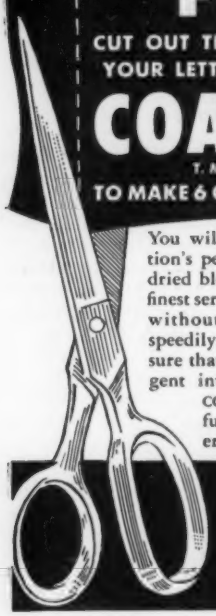

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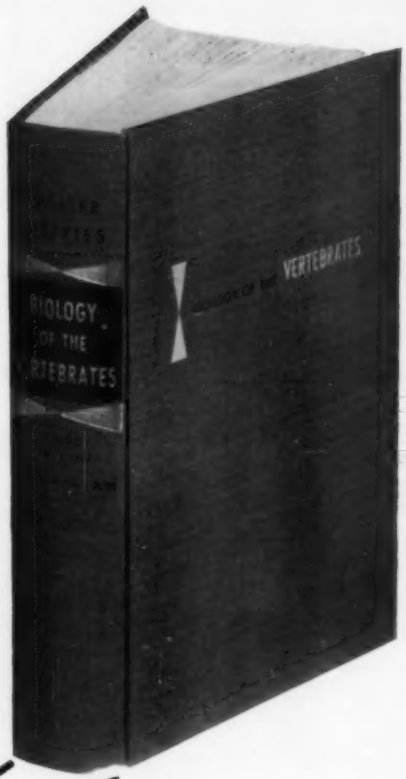

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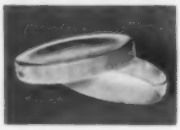

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